ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

10 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. V WASHINGTON, D. C., DECEMBER 20, 1915 No. 12

BIOLOGY OF APANTELES MILITARIS

By DANIEL G. TOWER,[1]
Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology

INTRODUCTION

The results herewith presented deal with *Apanteles militaris* Walsh, a braconid endoparasite of the army worm (*Heliophila unipuncta* Haw.). The series of experiments on which the main part of this paper is based was begun on September 29, 1914, at La Fayette, Ind. They were carried on in the laboratory, the parasitized caterpillars being kept in glass vials plugged with cotton and fed fresh corn leaves as required. The laboratory windows were left open, so as to make conditions as nearly like those outside as possible. During the few cold days which were experienced the laboratory was heated to the normal room temperature. During the first two weeks in August additional records were kept of the time spent in the cocoon by the parasites, and in these experiments cocoons were kept in tin salve boxes in an outdoor insectary. On November 16 a series of experiments was started indoors to determine whether or not this species is parthenogenetic, and conclusive results were obtained. The caterpillars used in the experiments were raised from eggs unless otherwise stated.

DESCRIPTION OF LIFE STAGES

THE EGG

The egg measures 0.09 to 0.10 mm. in length and 0.025 to 0.028 mm. in width. It is rounded at one end, more or less pointed at the other, and slightly curved, the rounded end bearing a distinct micropyle. Subsequent swelling of the egg during the growth of the embryo causes

[1] The writer wishes to acknowledge his indebtedness to Messrs. J. J. Davis and A. F. Satterthwait, of the Cereal and Forage Insect Field Station of the Bureau of Entomology at La Fayette, Ind., for many helpful suggestions and material, and to Messrs. J. A. Hyslop and G. G. Ainslie, of the Bureau of Entomology, for their interest and kindness in collecting material and data for him at their respective stations. He is also indebted to Messrs. A. B. Gahan and W. R. Walton, of the Bureau of Entomology, for the determination of specimens and for the drawings of the three larval stages, respectively.

the point of the smaller end to assume the appearance of a nipple-like prominence.

The number of eggs laid by a single individual was not obtained, nor were the eggs in the abdomen counted, hundreds having been present.

EMBRYONIC DEVELOPMENT

The average length of the egg stage is 5½ days. Individual records show that in some cases this may be shortened to 4¾ days or prolonged to more than 6⅛ days. From the hundreds of developing eggs examined it was determined that only one larva hatches from each egg.

Development progresses rapidly within the egg. At first little can be distinguished, except that the egg becomes strongly curved, increases in size, and becomes more opaque, owing to the formation of the germ band. When the egg is ready to hatch it has increased in size from 0.09 or 0.10 mm. in length to 0.66 or 0.70 mm., and proportionally in width. This great increase in size can possibly be explained by the fact that the egg is probably deficient in nutritive matter when laid and that this is absorbed from the blood of its host by the developing embryo.

When embryonic development has progressed sufficiently to show the form of the embryo, this is seen to be surrounded by a single embryonic envelope one cell layer deep which, according to Korschelt and Heider (3, p. 287),[1] is the serosa (Pl. L, fig. 1). Whether the amniotic and serosal envelopes are at first separate has not been determined. According to Graber's observations on Hymenoptera, as reviewed by Korschelt and Heider, it would seem that the two envelopes are separate at first but later become indistinguishably united. At the time of hatching, a portion of the cells of this so-called serosal envelope are cast out at the poles of the egg (Pl. L, fig. 2) and become a body of loose cells lying between the chorion and the embryo (Pl. L, fig. 3), which is now tightly inclosed by a layer of broad, flattened cells made up of the remaining cells of the envelope (Pl. L, fig. 3). This rapid division apparently indicates that this envelope was the product of the fused amnion and serosa, which now separate at hatching time, the loose mass of cells being of serosal origin and the remaining thin envelope the amnion surrounding the embryo. Henneguy (2, p. 336–337), however, discusses insects that have only one embryonic envelope and lists among these parasitic forms, vegetable or animal, of the Cynipidae, Pteromalidae, and probably Ichneumonidae. It will be interesting to note whether other investigators observe this splitting of the single embryonic envelope at hatching time.

The mandibles can be seen forming at an early stage, and their chitinization can be seen to progress until maturity is reached at hatching time.

[1] Reference is made by number to "Literature cited," p. 506–507.

The mouth opens into an enlarged cavity, the pharynx, this in turn opening posteriorly into a very narrow esophagus, and this into the stomach, which is a very long, narrow, tapering tube closed posteriorly. There are two Malpighian vessels, which lie parallel to the stomach, extending anteriorly about one-half the length of the larva.

The tracheal system has not been observed in the embryo. According to the observations of Weismann and Grasse, as reviewed by Korschelt and Heider (3, p. 334–335), the tracheal system forms early in the embryonic development of the Hymenoptera as compared with the lower forms of insects and usually contains air previous to hatching, this being obtained apparently from its tissues and body fluid. Seurat (7) states, however, from his study of *A. glomeratus*, that the tracheal system of this parasite, whose development is similar to that of *A. militaris*, is present, although he had not seen it, no doubt basing his statement on the fact that these organs, being ectodermal invaginations, are normally formed in the embryo.

The head of the mature embryo is of one segment and is readily distinguished by its large size, the presence of mandibles, two small tubercle-like antennæ, and the prominent brain lobes. A nervous system of 11 ganglia, not including the subesophageal ganglion, is visible. The segments of the body appear to be 10 in number, but subsequent development and growth in the first stage reveal 11 distinct segments.

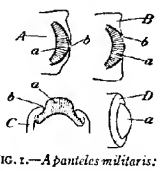


FIG. 1.—*Apanteles militaris: A, B, C,* Diagrammatic sectional views of the posterior end of the embryo, showing how the hypertrophied cells of the hind gut, which ultimately form the caudal vesicle, grow out through the anus. *D* shows an external view of this process. *a*, Mass of cells; *b*, anus. (Original.)

The caudal vesicle, which in the larval forms is a large sac at the end of the body, is seen forming as a solid mass of long, narrow cells in the posterior region of the abdomen (fig. 1, *a*). When first seen it lies inside the abdomen, but can be seen gradually to grow out through the anal opening (fig. 1, *b*), which becomes greatly distended. The stomach becomes lengthened and extends outside the body into the vesicle, its blind end being fastened to the inside wall of the vesicle posteriorly and ventrally. The Malpighian tubes also extend into the vesicle and open through its ventral surface near the end of the stomach.

HATCHING

The embryo at the time of hatching, as previously stated, lies tightly inclosed in the amniotic envelope surrounded by the loose mass of serosal cells, the whole being surrounded by the chorion. The embryo, which up to this time has been curled in the egg, now straightens itself out and by its struggles to escape, aided by the rapid swelling of the serosal cells, ruptures the chorion, which has become extremely thin, owing to the increase in the size of the egg, and escapes into the body of its host, still

tightly inclosed in the layer of thin, flat cells. The serosal cells are scattered through the body of the host. The chorion shrinks and probably finally dissolves. The young larvæ are now 0.7 mm. in length and start feeding, after cutting through the amnion in the mouth region. At this time the mass of cells which forms the caudal vesicle has grown out through the anal opening.

THE LARVA

FIRST INSTAR (Pl. L, fig. 5).—The first larval instar averages 3¼ days, the first molt taking place, on an average, 8¾ days after oviposition.

The larva grows rapidly, increasing in length approximately from 0.7 mm. at hatching to 3.5 mm. at the first molt.

The head is made up of 1 segment and the body appears to have 10, but in subsequent growth the tenth segment divides into 2, making 11 in all. There are no spines or hairs on the segments, except a few in the oral region. Owing to the rapid growth of the larva, the embryonic envelope in which it is inclosed becomes ruptured and gradually falls off, although portions of it may remain until the first molt takes place. The mandibles are constantly in motion, attacking the fat body of the host. This, together with the blood, is the food of the parasites during this stage and is drawn in by means of a sucking pharynx. The alimentary tract does not change, except to increase in size, it being still further lengthened as the caudal vesicle expands.

Immediately following hatching, the slender cells of the mass which protrudes from the distended anal opening are compressed lengthwise, so that they become broad, flat cells, thus immensely increasing their exterior and interior surfaces, and there is formed at the end of the larva a large sac, the caudal vesicle, the walls of which are made up of a layer of broad, thin cells (Pl. L, fig. 5). The two Malpighian vessels are drawn out into the caudal vesicle, their relative positions being the same as in the embryo.

The origin of this caudal vesicle and its functions in the two endoparasitic stages will be considered later.

The nervous system appears as in the embryo, its growth keeping pace with the growth of the larva.

No tracheal system is visible during this instar.

The heart can be seen forming in the early part of this instar. It lies dorsally and has nine pairs of valves, its lateral controlling muscles being readily seen. Anteriorly it narrows to an aorta which opens into the posterior region of the head. Instead of ending normally in the posterior end of the body, a rudimentary tube lying dorsally in the caudal vesicle connects with the heart (Pl. L, fig. 4). This tube extends posteriorly, opening in the dorsal posterior region of the caudal vesicle, and forms a channel through which the blood is sucked into the heart. When the heart commences to function, which it does during this stage, the blood,

having been drawn through the rudimentary tube into the heart, is there passed along by a series of wavelike motions into the head, the valves preventing the return of the blood. From here it circulates through the body in returning to the caudal vesicle, the walls of which it bathes before starting on a new cycle. A careful examination of the heart does not show that ostia are present; hence, the blood necessarily follows the course described above.

The silk glands can be distinguished early in this stage and lie on either side of the stomach as two straight tubes which meet anteriorly in the head and extend to the spinneret. As the end of this stage approaches, these glands begin to coil, taking on a wavy appearance.

SECOND INSTAR (Pl. L, fig. 6).—The second instar averages $5\frac{1}{2}$ days, terminating when the larva emerges from its host, for it molts at this time. During this stage the average increase in length is from 3.5 to 6 mm., although when a great many larvæ are present in a host their size may be reduced nearly one-half. The caudal vesicle normally during this stage reaches the length of 1 mm. (Pl. L, fig. 6).

The head of the larva is made up of 2 segments. The anterior one bears a few spines about the oral region and is much smaller than the posterior and almost wholly retractile in it. There are no notable characters or ornamentations on the segments of this larva. The body has 11 segments and is at first slightly darker than the first instar, but rapidly becomes more so as the fat body accumulates. The mouth parts are not developed, nor are those of the third instar ready for use, until the larva is ready to emerge from its host; hence, it is seen that only the blood and the solid matter contained in it are used for food during this stage. In older forms there are 7 hyaline areas protruding on each side of the body lying between the segments.

The silk glands grow rapidly, becoming more and more coiled and twisted, and are readily seen lying on either side of the alimentary tract, nearly filling the body cavity.

The heart and the circulation of the blood are the same as in the first instar.

The nervous system consists of the supraesophageal and the subesophageal brains and 11 ganglia with their branches, as in the first instar. In the early life of this stage the imaginal discs of the compound eyes are noticeable and appear to be in the first thoracic segment. The exhaustive studies of Seurat (7) show clearly that although other authors have thought that a portion of the prothorax entered into the composition of the head of the pupa, it is formed only from the head of the larva and that in the larval forms a portion of the head has simply been thrust back into the prothorax. Ventrally in the thoracic segments the three pairs of imaginal discs of the legs are present, and laterally in the mesothorax and metathorax those of the wings can be seen.

The mouth, pharynx, esophagus, and stomach have approximately the same form and relative positions as in the first instar. Owing to the fact that the blood of the host is green, the stomach content of the parasite at first takes on a greenish brown color which finally becomes a deep green, similar to the blood of the host, and later, at the end of the stage, this again becomes greenish brown.

During the last two days of this stage the anal opening, the diameter of which nearly equals that of the body, slowly contracts, and violent contractions of the longitudinal muscles of the stomach, which cause it to shorten, slowly draw the caudal vesicle in through the contracting anal opening. The Malpighian vessels are also drawn in by the contraction of the stomach and are now two-thirds as long as the larva. After the caudal vesicle has been drawn completely within the body, the anal opening contracts still further, and the anus is formed.

The tenidia of the tracheal system can be seen forming soon after the first molt. Those of the two main longitudinals and their anterior connecting branches are first visible, and there are 11 branching centers on each longitudinal from which arise branches sending tracheæ to all parts of the body, some even extending posteriorly into the caudal vesicle along the lateral walls and the stomach. Nine pairs of short, stublike branches are noticeable in the older larvæ, arising near the bases of the anterior nine pairs of dorsal branches of the main longitudinals. In the still older larvæ, those nearly ready to emerge, eight pairs of spiracles can be seen forming at the surface of the body, and these are connected with the first, and the third to ninth, inclusive, pairs of stublike branches previously mentioned, by tracheæ destitute of air. These become filled with air when the larva molts at emergence and the spiracles are uncovered and function. The spiracles that connect with the second pair of stublike branches do not form during this stage.

After the caudal vesicle has been drawn in, the larva is ready to emerge from its host. The mandibles of the third instar, which are now developed and protrude, slowly cut and tear through the muscles and skin of the host as the larva presses its head against the body walls of the caterpillar and moves them backward and forward. When a slit has been made of sufficient size the larva squeezes through the opening, molting the previously loosened skin as it emerges. During this process the caterpillar lies quietly as though paralyzed. About the time the parasites have nearly finished their cocoons, it usually revives enough to crawl away.

THIRD INSTAR (Pl. L, fig. 7).—The third instar lasts from the emergence from the host until pupation, the time being approximately 2¼ days.

The newly emerged larva is light green in color. It is covered with minute spines, with a number of short black spines somewhat irregularly

placed on the segments; also about the mouth there are a few hyaline spines. The brown-colored compound eyes are very noticeable and appear to be, as in the second stage, in the prothorax. The segmentation of the body is apparent and the eight pairs of spiracles are plainly visible (Pl. L, fig. 7). The mouth parts are at the extremity of the head and are composed of labrum, mandibles, maxillæ (bearing the rudimentary maxillary palpi), and the labium (bearing the rudimentary labial palpi). Laterally the seven hyaline protruding areas form an irregular, conspicuous, longitudinal ridge on either side of the body.

When the larva has emerged for about two-thirds of its length, it stops and commences to spin its cocoon. The silk comes from the two orifices of the spinneret situated at the base of the labium. The cocoon is spun in two parts, the outer part loosely and the inner compactly. The first few threads spun are fastened to the ventral side of the body, after which a series of large loops are made, the silken thread being drawn out and fastened to the top of the loop below. These extend up the ventral side laterally and over the head of the larva as far back as it can bend. The larva now draws its anal end out of the host, reverses its position in the partly spun outer cocoon, and spins the remaining side and end. The inner or thin, dense cocoon is now spun by a series of long, narrow, longitudinal and diagonal loops. The tough silken cocoon is encircled near one end, or sometimes at both, by a thinner, narrow area, through which the adult parasite easily cuts, removing a caplike portion, the end of the cocoon, as it emerges.

At the end of the first day or the beginning of the second the connection between the stomach and proctodeum is opened and the accumulated waste is voided, being deposited at the anal end of the cocoon. When pupation takes place, the last larval skin is molted and pushed to the anal end of the cocoon and lies over the waste. Previous to pupation, the constriction between the thorax and abdomen, which results in the cephalization of the first abdominal segment, is distinctly seen.

PUPA AND ADULT

The pupal stage averages from 8½ to 9½ days.

The pupa is light cream yellow and lends the same color to the cocoon. The eyes and ocelli appear as brown spots. Later, the chitin in the head and thoracic region commences to darken, closely followed by that of the abdomen. When the adult becomes active in the cocoon, the pupal skin is kicked off, and the area of thin silk is cut through by the mandibles, the end, or cap, of the cocoon being pushed off by the emerging adults. As soon as the adult is out of the cocoon, it passes a quantity of waste, cleans itself, and straightens and dries its wings.

LENGTH OF LIFE CYCLE

The total length of the life cycle, as obtained in the series of experiments carried from the last of September to the last of October, averaged 25 days. A series of experiments conducted during the first two weeks of August to determine the time spent by the third instar and pupa in the cocoon varied from 5 to 7 days, as compared with 11 to 12 days during September and October. This great reduction in the time spent in these periods of development raises the question whether or not the time spent in the host would not be shortened under summer conditions. Unfortunately, this point could not be determined; but considering that the duration of the larval life of the army worm varies from 20 to 30 days, according to Slingerland (8), it seems not unlikely that the length of the egg and internal larval stages would vary correspondingly with the life of the host.

COPULATION

The following observations were made on these insects confined in test tubes and lantern-globe cages. The male pursued the female, caressing her with his antennæ, often mounting her posteriorly and, thrusting his abdomen forward, bringing the ventral surface in contact with that of the female. Once union had taken place the male folded his wings and drew his legs close to his body, holding on to the female solely by his genitalia. It was noticed that in the case of a number of males and females confined in test tubes for several days, copulation continued to take place day after day with unabated vigor.

OVIPOSITION

The parasite apparently recognizes the host on touching it with its antennæ, and following such recognition the ovipositor is bent beneath the thorax, sometimes slowly but usually quickly, and is then rapidly thrust into the caterpillar. This being done, the parasite folds its wings and draws its legs up close to its body, holding on to the caterpillar solely by its ovipositor, this no doubt being done to protect itself from the attacks of its host. During the process of oviposition the caterpillar may throw itself about violently, but rarely dislodges the parasite.

Of the number of apparent ovipositions in larvæ of the third, fourth, and fifth stages, one-sixth of those which took place in the third, one-fifth of those in the fourth, and one-half of those in the fifth stage were unsuccessful. Usually the parasite larvæ emerge after the caterpillar is full grown, as observed in the case of larvæ collected in the field and those parasitized in the laboratory under artificial conditions, but in one instance where the parasite oviposited in a caterpillar of the third stage the parasite larvæ issued during the fifth stage.

Parasites readily attempted to oviposit in caterpillars of the fifth and sixth stages, but were apparently unsuccessful, on account of the tough-

ness of the skin, except in newly-molted fifth-stage larvæ. In such cases they would run along the back of the host, jabbing with the ovipositor but never succeeding in puncturing the skin.

The eggs, when dissected from the body of a caterpillar immediately following oviposition, are found to be separate.

Oviposition in the field under natural conditions resulted in the following numbers of cocoons collected from single hosts: 56, 90, 71, 79, 90, 7, 113, and 66. In the laboratory from 8 to 72 eggs were deposited in one oviposition of less than one second, and in one case of four ovipositions 210 eggs were deposited in the same host. The extreme rapidity of oviposition is apparently due to the activity of the caterpillar, which usually immediately recognizes its enemy, rapidly smearing her with saliva and often biting her.

PARTHENOGENESIS

During November and December a number of experiments were conducted in the laboratory to determine whether parthenogenesis takes place. Unfertilized females were obtained from separate cocoons and were allowed to oviposit in small caterpillars, which they readily did. Males emerged from all the cocoons of *A. militaris* originating from these caterpillars, clearly showing that this species is parthenogenetic and indicating that unfertilized females give rise to a generation of males.

FEEDING EXPERIMENTS AND LONGEVITY

Adults which emerged on August 14 were confined in a lantern-globe cage in which grass was growing. They were fed on a mixture of honey and water, this being sprayed in minute droplets on the grass and walls of the cage. The adults were of both sexes and were kept alive for some time, the last one dying on September 1.

One female used in oviposition experiments was kept alive for eight days in a test tube, being fed honey, and another under the same conditions lived for seven days.

On November 6 and 7 a large number of newly emerged males were confined and fed in two lantern-globe cages indoors, as described above. These males were not allowed to copulate, and many lived until the first of December, the last dying on December 9 and 10.

WINTERING FORMS

All attempts at this station (La Fayette, Ind.) to winter this parasite under various conditions while in the cocoon have been unsuccessful. Mr. G. G. Ainslie, stationed at Nashville, Tenn., found this year (1915) that the army worm passed the winter there as young larvæ and, further, that specimens under observation were parasitized in the fall, for the parasites completed their growth and emerged this spring. Again, according to Gibson (1, p. 27), the army worm winters in Canada as

young larvæ beneath tufts of grass. Considering the data at hand, the theory is advanced that in the North the parasites winter as partly developed forms in immature larvæ, while in the South they no doubt also winter while in the cocoon.

ORIGIN AND FUNCTION OF THE CAUDAL VESICLE

The following is a summary of the results of the studies of Weissenberg and Seurat, together with the observations made by the writer, on the origin and function of the caudal vesicle, obtained mainly from experiments with hymenopterous endoparasites.

As Seurat's (7) and Weissenberg's (9) papers both deal with *A. glomeratus*, the caudal vesicle of which originates and functions identically as does that of *A. militaris*, the results of their studies are applicable to *A. militaris*. Weissenberg's paper, being the more exhaustive and, in addition, containing studies of the larva of this parasite in comparison with others less highly specialized, is used as a basis for this summary.

Observing the beginning of growth and the subsequent expansion of the caudal vesicle, the writer supposed that the entire proctodeum evaginated and turned inside out, but the careful histological studies of *A. glomeratus* by Weissenberg show that only a portion of the proctodeum through rapid growth becomes specialized to form the vesicle, while the remainder becomes temporarily atrophied. According to Weissenberg, the vesicle is formed by the rapid growth and elongation of the cells of the proctodeum which form the posterior end of the plug at the posterior end of the stomach, together with those adjacent cells at the anterior end of the proctodeum which surround the opening of the larval Malpighian tubules and extend posteriorly a short distance to the rudiments of the adult Malpighian tubules. The mass of elongated cells thus formed grows out through the anal opening of the embryo, and immediately following hatching these elongated cells are compressed lengthwise, so that their long axis becomes their short one, resulting in broad, flat cells joined edge to edge to form the thin wall of the caudal vesicle. During the rapid growth of these cells in the pyloric region the remainder of the proctodeum becomes atrophied and stays so until the caudal vesicle is drawn in. At this time parts specialized for endoparasitic life are reduced, and the atrophied parts grow rapidly, the whole approaching the normal proctodeal development of a free-living hymenopterous larva, previous to pupation.

Weissenberg next compares the origin and cellular structure of the caudal vesicle of *A. glomeratus* with that of the caudal appendage of the endoparasitic larval form of an undetermined species of Macrocentrus, and shows them to be homologous. In *Macrocentrus* sp., however, the cells always remain as a mass of long, slender cells protruding through the anal opening, a vesicle never being formed. The early stage of the

species of Macrocentrus studied was equipped with a tracheal system, while the corresponding stage of *A. glomeratus* was not. The conclusion is drawn that the vesicle functions as a blood gill in *A. glomeratus*, since all the blood necessarily pours through this vesicle, bathing its walls, while in *Macrocentrus* sp., which possesses a tracheal system, such an adaptation is not necessary.

An unknown species of the genus Limneria, parasitic on *Plutella cruciferarum* Zell., is next introduced for comparison by Weissenberg. In this parasite the portion of the proctodeum homologous with those of the two preceding larvæ discussed is not so well developed, for while pseudopod-like structures extend into the anal lumen, they do not protrude through the anal opening, which, however, is nevertheless very large. In this species it is clearly shown that the cells of these pseudopod-like structures completely correspond histologically with those of the larval Malpighian tubules. In a similar manner these specialized portions of the proctodeum of the two species last discussed are reduced and the portions retarded grow rapidly, approaching the normal proctodeal development of free-living larvæ before pupating, the normal proctodeal development of *Hemiteles fulvipes*, an ectoparasite of *A. glomeratus*, being used in comparison to illustrate this.

In the last analysis it is seen that the cells of these proctodeal appendages of the three endoparasitic larvæ considered are histologically allied with the cells of the larval Malpighian vessels, and with this in mind Weissenberg brings out clearly the idea that these proctodeal organs have also an excretory function and credits Kulagin (4, 5) with first suggesting this from results obtained from his injection experiments. Weissenberg further thinks that the excretory apparatus has undergone a superficial enlargement, owing to the active metabolism characteristic of this group, and that as excretory products in general are poisonous, it would seem natural to find here an adaptation by which they can be eliminated. His concluding argument is that in *A. glomeratus*, *Macrocentrus* sp., and *Limneria* sp. the development of the larval Malpighian vessels forms an ascending series, they being only rudimentary in *A. glomeratus* in comparison with the well-developed ones found in *Limneria* sp., while the proctodeal adaptations form a descending series, being most highly specialized and developed in *A. glomeratus* and only partly so in *Limneria* sp.

From the facts presented above and this study of *A. militaris*, the author concludes that the caudal vesicle is primarily an excretory organ and that the function of respiration is secondary. The following observations seem still further to strengthen this conclusion. The caudal vesicle functions from approximately the beginning of feeding to its close, and the portion of the first skin molted which covers the vesicle becomes greatly swollen in the second stage with a liquid content until finally it is ruptured. Further, the food of the larva is mainly the already digested solid parts

of the blood of the host, these being retained in the stomach during endoparasitic life, while the liquid parts, which are in excess, together with the by-products of anabolism and katabolism formed in the body of the rapidly developing larva, are eliminated by means of this enlarged adaptive excretory organ, which is bathed by the blood at each cycle. These by-products are doubtless eliminated from the body of the host, as are its own, by the Malpighian vessels. The caudal vesicle no doubt respires, this action taking place by osmosis, as is generally considered to be the case in endoparasites having a closed tracheal system. Whether respiration is more rapid through the walls of the caudal vesicle or whether they are especially adapted for it can not be positively stated, although Weissenberg, as stated previously, thinks that the vesicle functions as a blood gill. Again, that this portion of the body wall of the larva is apparently the thinnest and least chitinized is quite evident; therefore, it would not seem unreasonable to suppose that respiration takes place to a large degree through this area and that the air is carried mechanically throughout the body of the larva by the blood and is taken up from it to fill the closed tracheal system when it develops in the second instar.

Seurat's theory (7) that the essential function of the caudal vesicle is that of locomotion is no doubt incorrect, for careful observations of the movements of the larva show that the vesicle, because of its large size, is actually a hindrance to the larva in moving about in its host. Weissenberg (9) has also shown that the caudal vesicle is not homologous with the tail-like organs generally considered to be locomotor appendages which occur in various endoparasites, for both these organs are present in the larva of *Macrocentrus* sp. studied.

An additional point brought out by Weissenberg is that the caudal vesicle is an adaptation of the biophagous larva for its mode of life, for the necrophagous larva does not have it, and these adaptations arise from a biophagous mode of life in contrast with the necrophagous rather than from an endoparasitic life in contrast with an ectoparasitic life, as has been previously supposed.

LITERATURE CITED

(1) GIBSON, Arthur.
1912. Cutworms and army-worms. Canada Dept. Agr. Div. Ent. Bul. 3 (Exp. Farms Bul. 70), 29 p., 10 fig., 1 pl.

(2) HENNEGUY, L. F.
1904. Les insectes, Morphologie—Reproduction—Embryogènie . . . 804 p., illus., 4 col. pl. Index bibliographique, p. 695–756.

(3) KORSCHELT, Eugen, and HEIDER, Karl.
1899. Textbook of the Embryology of Invertebrates . . . Translated from the German . . . v. 3, London, New York.

(4) KULAGIN, Nicolaus.
1892. Notice pour servir à l'histoire du développement des hyménoptères parasites. *In* Cong. Internat. Zool. 2e Sess. Moscou, pt. 1, p. 253–277.

(5) KULAGIN, Nicolaus.
1892. Zur Entwicklungsgeschichte der parasitischen Hautflügler. (Vorläufige Mittheilung.) *In* Zool. Anz., Jahrg. 15, No. 385, p. 85-87.

(6) PACKARD, A. S., Jr.
1898. Text-Book of Entomology, including the Anatomy, Physiology, Embryology and Metamorphoses of Insects. 729 p., illus. New York.

(7) SEURAT, L. G.
1899. Contributions à l'étude des Hyménoptères entomophages. *In* Ann. Sci. Nat. Zool., s. 8, t. 10, no. 1/3, p. 1-159, 16 fig., 5 pl.

(8) SLINGERLAND, M. V.
1897. The army-worm in New York. N. Y. Cornell Agr. Exp. Sta. Bul. 133, p. 231-258, fig. 68-72.

(9) WEISSENBERG, Richard.
1909. Zur Biologie und Morphologie endoparasitisch lebender Hymenopterenlarven (Braconiden und Ichneumoniden). *In* Sitzber. Gesell. Naturf. Freunde, Berlin, Jahrg. 1909, No. 1, p. 1-28, 8 fig.

PLATE I.

Apanteles militaris:

Fig. 1.—Diagrammatic drawing showing the embryo inclosed by the fused amniotic and serosal envelopes. *as*, Fused envelopes; *c*, chorion; *cv*, caudal vesicle; *h*, head.

Fig. 2.—Diagrammatic drawing showing the fused envelopes dividing into their two parts, the serosal cells being grouped at each pole. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

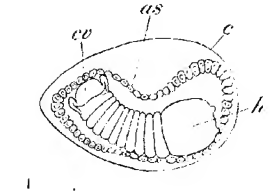
Fig. 3.—Diagrammatic drawing showing the egg ready to hatch, the serosal cells having become a loose mass and the embryo straightened out in the egg. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

Fig. 4.—Diagrammatic drawing of the larva during its first molt. *b*, Brain lobes; *cv*, caudal vesicle; *h*, head; *ht*, heart; *m*, molted skin; *mp*, Malpighian tubes; *o*, esophagus; *p*, pharynx; *sg*, silk glands; *st*, stomach; arrows indicate the blood cycle; *t*, rudimentary tube in the caudal vesicle connecting with the heart.

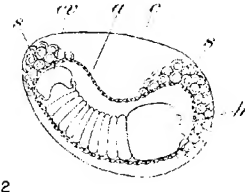
Fig. 5.—First instar. *cv*, Caudal vesicle.

Fig. 6.—Second instar. *cv*, Caudal vesicle.

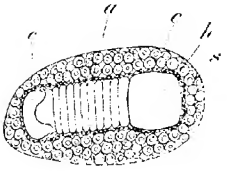
Fig. 7.—Third instar, showing the position of the spiracles and the caudal vesicle withdrawn.



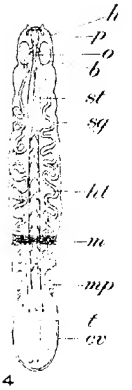
1



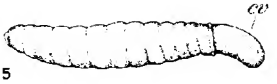
2



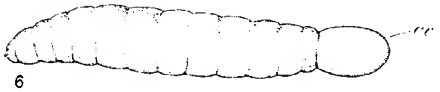
3



4



5



6

7

RESPIRATION EXPERIMENTS WITH SWEET POTATOES

By HEINRICH HASSELBRING and LON A. HAWKINS,

Plant Physiologists, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry

INTRODUCTION

In 1882 Müller (7),[1] in the course of his classical researches on the accumulation of sugar in plant organs at low temperatures, observed that potatoes (*Solanum tuberosum*) which had been kept for a time at 0° C., and whose sugar content had in consequence been greatly increased, respired much more energetically than potatoes of lower sugar content. Even before the experiments of Müller, a number of analogous facts were known, all indicating that the respiratory energy of plants is a function of their carbohydrate content. Thus, isolated rootlets and seedlings deprived of their cotyledons show a rapid decrease in their respiration on account of the lack of plastic material normally furnished by the cotyledons (12). In etiolated seedlings the respiration curve rises at first as the food substances in the cotyledons or endosperm become available, and after passing a maximum falls gradually with the exhaustion of the food reserve (6, 11). The respiration of isolated leafy shoots kept in the dark sinks rapidly also, but if such shoots are exposed for a time to sunlight their respiration is considerably increased (1, 2). So also, if the carbohydrate content of etiolated leaves, shoots, or seedlings is increased by an immersion of the parts in sugar solutions, respiration is greatly stimulated, although Palladine attributes the increased respiration partly to the formation of active proteins produced under conditions of favorable carbohydrate nutrition (5, 8, 9).

Since the sugar content of sweet potatoes (*Ipomoea batatas*) changes greatly in storage, it appeared not unlikely in view of the foregoing facts that their respiratory activity would show corresponding changes at different seasons. The experiments described in the following pages were performed in order to ascertain whether any such correlation exists between the seasonal changes in the sugar content of sweet potatoes and their respiratory activity, and incidentally to determine if possible whether the monosaccharids or the disaccharids of the sweet potato furnish the chief material for respiration. The roots were taken from the lots stored for experimental purposes under the conditions described by the writers in a former paper (4). The details are given in connection with the descriptions of the individual experiments. The respiration

[1] Reference is made by number to "Literature cited," p. 517.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bk

Vol. V, No. 12
Dec. 20, 1915
G—68

experiments were all carried out at 30° C. This temperature was chosen in order to study the respiration of the sweet potatoes under conditions similar to those to which the freshly dug roots are subjected during the curing process, which consists essentially in keeping them at a temperature in the neighborhood of 30° C. for about 10 days.

EXPERIMENTAL METHODS

The methods employed in the experiments require but little description. The sweet potatoes were placed in a large receptacle in an ordinary water-jacketed incubator, which was kept at a temperature of 30° C. A current of air having the same temperature and freed from carbon dioxid was drawn through the receptacle at the rate of 40 to 50 liters per hour. The carbon dioxid of respiration was collected in approximately one-half normal potassium-hydroxid solution, whose titre for pure potassium hydroxid had been determined. The absorption was effected by means of Reisette flasks. At the end of every 24-hour period the carbon dioxid in the Reisette flask was precipitated by means of an excess of barium chlorid, and the residual potassium hydroxid was determined by titration with normal or half-normal hydrochloric acid.

About 2 to 3 kgm. of sweet potatoes were used in each experiment. At the beginning of the experiment the sugar content was determined in a collateral sample of 3 to 4 kgm. from the same lot. At the end of each experiment all the sweet potatoes which had been used for that experiment were ground and sampled for determinations of sugar and moisture. The figures giving the sugar determinations are averages of five samples from each lot. The directly reducing sugar was calculated as glucose. The soluble carbohydrates yielding reducing sugar after inversion were calculated as cane sugar, which is the most abundant disaccharid present in the sweet potato. Jersey Big Stem sweet potatoes were used in all the experiments.

EXPERIMENTAL DATA

The results of all the experiments are collected in Table I. The percentages of total sugar (as glucose), cane sugar, and reducing sugar (as glucose) in the collateral sample taken at the beginning of each experiment, and in the experimental sweet potatoes at the end of the experiment, are given at the head of the table. These figures were in each case calculated for sweet potatoes of the water content of the collateral sample—i. e., the assumed original water content of the experimental sweet potatoes. The carbon-dioxid output is given in milligrams per kilogram per hour for each day. In the calculation the loss of weight of the sweet potatoes during the experiment was taken into consideration and was distributed uniformly over the period. At

the end of Table I is given the gain or loss of reducing sugar, calculated from the analytical data, and the glucose equivalent of the total carbon dioxid generated during each experiment, as actually determined. The percentages of reducing sugar in the sweet potatoes at the end of each experiment, without correction for changes in water content, were as follows: First experiment, 1.24 per cent; second, 1.22 per cent; third, 1.39 per cent; fourth, 0.91 per cent; fifth, 0.71 per cent; sixth, 0.67 per cent; seventh, 0.69 per cent. The experiments themselves will be described individually.

TABLE I.—*Composition and carbon-dioxid output of sweet potatoes at different times of the year*

Item.	Period.	Experiment 1, Oct. 21 to Nov. 5.	Experiment 2, Nov. 7 to Nov. 17.	Experiment 3, Dec. 9 to Dec. 19.	Experiment 4, Jan. 4 to Jan. 15.	Experiment 5, Mar. 26 to Apr. 5.	Experiment 6, Apr. 16 to Apr. 26.	Experiment 7, June 1 to June 11.
Total sugar (as glucose), per cent.	At beginning of experiment.	2.62	5.80	8.99	6.22	7.03	7.41	7.30
	At end of experiment.	5.38	5.08	8.42	5.82	7.45	7.40	7.20
Cane sugar, per cent.	At beginning of experiment.	1.60	3.41	6.58	4.63	5.82	6.17	6.08
	At end of experiment.	3.95	3.72	6.74	4.71	6.42	6.41	6.21
Reducing sugar, per cent.	At beginning of experiment.	.94	2.21	2.06	1.35	.90	.92	.90
	At end of experiment.	1.23	1.18	1.35	.87	.70	.67	.68
Daily rate of carbon-dioxid output, mgm. per kgm. per hour.	1st day	27.7	73.9	138.2	49.1	50.9	46.6	47.5
	2d day	24.9	82.1	144.9	56.0	44.9	49.4	42.4
	3d day	36.5	70.9	116.4	54.1	47.0	48.4	43.4
	4th day	35.7	60.0	101.8	48.4	46.8	46.4	46.2
	5th day	37.1	51.8	92.9	48.2	47.5	43.3	42.9
	6th day	31.8	45.9	90.4	44.0	47.0	42.0	43.1
	7th day	41.7	40.4	84.9	42.6	46.1	42.1	40.7
	8th day	34.3	39.1	83.3	40.7	46.5	39.3	42.2
	9th day	31.6	34.8	76.7	39.8	44.9	40.8	41.0
	10th day	29.8	32.8	79.9	38.9	41.1	39.3	44.1
	11th day	28.8	.....	.....	41.4	.....	.....	.....
	12th day	28.5	.....	.....	.....	.....	.....	.....
	13th day	24.9	.....	.....	.....	.....	.....	.....
	14th day	31.7	.....	.....	.....	.....	.....	.....
	15th day	29.9	.....	.....	.....	.....	.....	.....
Increment in reducing sugar calculated from the analytical data, gm.	.....	9.77	−31.69	−17.35	−10.03	− 3.40	− 5.14	− 2.50
Loss of reducing sugar equivalent to the carbon dioxid evolved, gm.	.....	27.45	25.85	35.18	15.80	11.73	13.41	7.33

EXPERIMENT 1.—In this experiment 3,576.5 gm. of sweet potatoes were used. These were dug on October 20. The experiment was begun on the following day and continued until November 5. During that period the cane-sugar content rose from 1.60 to 3.95 per cent and the invert-sugar content from 0.94 to 1.23 per cent. The respiration rose somewhat during the first half of the period and then fell to a nearly uniform rate of approximately 28 mgm. per kilogram per hour. The rise at first, which was observed in nearly all the other experiments also, may in part be attributed to the rise of the temperature of

the sweet potatoes when they were put into the incubator. Although there is a marked increase in both cane sugar and reducing sugar in the sweet potatoes, there is no evident general rise in the respiratory activity corresponding to the increase in the sugar content. During the course of the experiment the equivalent of 27.45 gm. of glucose was given off by the sweet potatoes as carbon dioxid, yet during this period 9.77 gm. of reducing sugar accumulated in them. The loss of weight of the sweet potatoes was 77 gm.

EXPERIMENT 2.—The sweet potatoes used in the second experiment were of the same lot as those of the first, but they had stood in the laboratory at a temperature of about 20° C. until November 7. The weight of the roots used for the experiment was 3,029.8 gm. The loss of weight was 138.8 gm. The percentage of cane sugar rose slightly, but the reducing sugar fell from 2.21 to 1.18 per cent. The respiration was high at first and fell gradually, apparently with the decreasing percentage of reducing sugar. It is clear that if in this case the lowering of the respiratory activity is due to the decrease of sugar, the effect must be wholly attributed to the change in the invert-sugar content, since the cane sugar, so far as may be judged from the analysis of the collateral sample, remained stationary or even rose slightly. The changes in the quantity of reducing sugar in these sweet potatoes are of special interest, for here the quantity of reducing sugar lost, according to calculations based on the analytical data, is greater than that lost through respiration as calculated from the quantity of carbon dioxid evolved. It seems, therefore, that a portion of the reducing sugar was used for other processes than respiration, possibly for the production of cane sugar.

EXPERIMENT 3.—The sweet potatoes used in the third experiment had been subjected to the regular curing process and had thereafter been kept in cold storage at a temperature of 6° to 7° C. from November 8 to December 9. The roots used in the experiment weighed 2,207.2 gm., and their loss of weight was 184.2 gm. As a result of the exposure to low temperature, the sugar content of these sweet potatoes was higher than of those used in any of the other experiments. The respiration of these chilled roots was also very high, but sank rapidly toward the end of the experiment. The quantity of reducing sugar equivalent to the carbon dioxid evolved in respiration was greater than the apparent decrease calculated from the analytical data.

EXPERIMENTS 4, 5, 6, AND 7.—The remaining experiments all present a certain uniformity and may be described together. The sweet potatoes used in these experiments were cured in the usual manner and were thereafter stored at a temperature of 12° to 15° C., until the dates on which they were used. The weights of the sweet potatoes used in the different experiments were 1,984, 1,577.5, 1,898.5, and 1,054.5 gm., respectively. The corresponding losses were 143, 56.5, 59.3, and 40.8

gm. The sugar content of these lots was remarkably uniform. Only the lot used in the fourth experiment was lower in cane sugar and higher in reducing sugar than the rest. In spite of this difference, the respiration in all cases was practically the same, beginning in the neighborhood of 50 mgm. per kilogram per hour and falling to about 40 mgm. toward the end of the experiments. In all cases the glucose equivalent of the carbon dioxid generated was higher than the loss of reducing sugar calculated from the analytical data.

DISCUSSION OF RESULTS

A comparison of the sugar content of the sweet potatoes in the different experiments with the respiration of the roots shows that no general correlation is evident between the total sugar content and the respiratory activity. It is true, indeed, that the roots having the highest sugar content (third experiment) also had the highest respiration, but these sweet potatoes had been subjected to low temperature for a month, and it is likely that such treatment induces other changes than those indicated by the carbohydrate transformations, for sweet potatoes thus treated become subject to the attacks of certain fungi which ordinarily do not readily invade the tissues. Moreover, it appears from experiments of Palladine (10) that, with a plentiful supply of carbohydrates present, plant organs which have been exposed for a time to low temperature respire more energetically when brought into a high temperature than those which have been continually kept at the higher temperature. Furthermore, the carbon-dioxid production in the third experiment fell off rapidly until it was no greater than that at the beginning of the second experiment, but the total sugar content of the sweet potatoes in the third experiment remained at all times much higher than that of the roots in the second experiment. The other experiments also show no correlation between the total sugar content of the sweet potato and the respiratory activity. Thus, the roots in the second experiment were low in total sugar, but had a high respiration, while those in the fifth, sixth, and seventh experiments had a comparatively high sugar content and low respiration. It is possible that irregularities in the size and shape of the sweet potatoes might account for differences in respiratory activity, but these sources of error were avoided as far as possible by the selection of fairly uniform roots. It is therefore unlikely that great differences in respiratory activity can be attributed to these factors.

While there appears to be no evident correlation between the total sugar content and the respiratory activity, the case is different when the reducing sugar alone is considered. Here there is evidence of a general parallelism, which, however, is easily obscured by other factors. This correlation is perhaps most clearly brought out by the gradual fall of the respiration, with the disappearance of the reducing sugar in the indi-

vidual experiments. The first experiment, however, is in marked contrast to the others in this respect, for, although the sugar content of these sweet potatoes rose from 0.94 to 1.23 per cent, there was no corresponding rise in the respiration. The parallelism between the respiration and the sugar content is less marked when the different experiments are compared. Thus, the roots in the second experiment contained approximately the same percentage of reducing sugar as those in the third, yet the respiration was much lower in the second. This fact, as has been pointed out, may probably be ascribed to the treatment to which the sweet potatoes had been subjected before the experiment. It is evident on the whole that the respiratory activity of the sweet potatoes is as greatly influenced by seasonal changes and environmental factors to which they have been exposed as by the sugar content. It is clear, of course, that with the exhaustion of the carbohydrates immediately utilized in respiration, the rate of respiration will fall, as in the case of seedlings grown continually in the dark, but it seems that an increase of the available carbohydrate supply does not necessarily entail a continued increase in the respiratory activity. That there is sufficient sugar present in sweet potatoes, as well as in plant organs generally, to support a more active respiration than usually takes place, is shown by the increased respiration as a result of wounding. Table II gives the carbon-dioxid output per kilogram per hour of two lots of sweet potatoes for a short period before and after they were split lengthwise.

TABLE II.—*Carbon-dioxid output in milligrams per kilogram per hour of two lots of sweet potatoes for a short period before and after being split lengthwise*

Before roots were split.			After roots were split.		
Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.	Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.
	Mgm.	*Mgm.*		*Mgm.*	*Mgm.*
1	4.4	42.7	7	9.3	60.0
2	4.1	39.2	8	6.9	50.8
3	4.7	36.3	9	7.2	52.7
4	5.4	35.4	10	7.2	70.7(?)
5	5.7	32.8	11	7.4	56.4
6	5.6	29.8	12	7.3	54.5
			13	7.6	52.5

The great increase in respiration after the sixth day, when the roots were split, shows that there was sufficient sugar present to support a more energetic respiration than that which took place in the whole roots, but that other limiting factors than the sugar supply determined the rate of respiration.

In the consideration of the question of the relative availability of the monosaccharids and the disaccharids as sources of material for respira-

tion, a certain allowance should perhaps be made for the nonconformity of samples, since the sugar content of the sweet potatoes at the beginning of each experiment was necessarily determined in collateral samples. Nevertheless, two facts appear evident. During the course of the experiments there was no diminution, but, on the contrary, an increase, in the quantity of cane sugar present in the sweet potatoes, while there was a marked decrease in the reducing sugar in all the experiments except the first.

The rise in the cane-sugar content of the sweet potatoes is most marked in the first experiment, but in this case the rapid change is simply an example of the generally observed manifestation that the sugar content of sweet potatoes is low while they are in the ground and rises rapidly immediately after they have been dug. In all the other experiments, although the increase is small (from 0.08 to 0.6 per cent), the differences all point in one direction. It seems clear, therefore, that there was at any rate no decrease in the cane-sugar content of the sweet potatoes during the course of the experiments.

This fact indicates that at 30° C. the cane sugar is reformed as rapidly as it is used for respiration or that it does not function in the respiratory processes, at least while other carbohydrates are present in abundance. Which of these possibilities occurs can not with certainty be determined from the data. A number of relative facts, however, seem to point to a rather high degree of stability of the cane sugar in the sweet potato, in so far as the processes of respiration are concerned. It has been found as the result of many analyses that at low temperatures (5° C.) there is an extensive accumulation of cane sugar in the sweet potato and that this increase of sugar takes place at the expense of the starch, which disappears correspondingly. At higher temperatures (15° to 20° C.) the accumulation of cane sugar is much less extensive and, in fact, does not proceed beyond a certain maximum, which, during the season's storage, is reached in March or early April. After the period of sugar formation the starch content of the sweet potatoes remains fairly constant, for the quantity of starch which disappears in respiration compared with the quantity used in the formation of sugar is so small that in view of individual differences among sweet potatoes and the errors of manipulation it has not been possible to determine the changes in starch content in connection with respiration in experiments carried on for short periods of time.

These facts seem to indicate that at higher temperatures the production of cane sugar is depressed. We should therefore expect that if sweet potatoes which have been stored at 15° to 20° C. until the cane-sugar content has attained an equilibrium (March to April) are subjected to a temperature of 30°, the production of cane sugar would be still further retarded or even inhibited. At the same time the rate of respiration is accelerated.

If no more cane sugar is formed and its utilization is hastened, we should expect a reduction in the quantity of cane sugar, at least in the experiments at the end of the season, if that substance is used in respiration. Such a reduction, however, occurs neither at the end of the season nor at any other time. It appears not unlikely, therefore, that the cane sugar in the sweet potato is relatively stable, with respect to the respiratory processes.

Although there was no diminution of cane sugar in the sweet potatoes used in these experiments, there was a marked decrease in the reducing sugar in all cases except the first. The first experiment, in which freshly dug roots were used, is exceptional for the reason mentioned above. It shows that in freshly dug roots the processes of sugar formation are so rapid that even at 30° C. sugar is formed faster than it is used in respiration. In this instance an amount of carbon dioxid equivalent to 27.45 gm. of glucose was evolved during the experiment, and in addition to this there was an increment of 9.77 gm. of reducing sugar, as calculated from the percentages present in the sweet potatoes at the beginning and at the end of the experiment. In all the other experiments there was a decrease of reducing sugar—i. e., the quantity of reducing sugar which had accumulated while the sweet potatoes were stored at low temperatures was diminished when the roots were subsequently exposed to a higher temperature. It is reasonable to infer that the sugar was utilized in respiration, but it will be observed that in all but the first and second experiments the loss of reducing sugar calculated from the percentages at the beginning and at the end of the experiments accounts only for a portion of the sugar equivalent to the quantity of carbon dioxid evolved. The deficiency is no doubt made up by the transformation of starch, for, as Deleano (3) found in the case of grape leaves cut from the vines, the starch functions readily in the respiratory processes. In the sweet potato the starch appears to be even more readily available than the cane sugar. In the second experiment, where the invert-sugar content was high at the beginning of the experiment, a synthesis of other carbohydrates may perhaps be assumed.

CONCLUSIONS

The experiments described in this paper seem to indicate that there is no general correlation between the total sugar content of the sweet potato and its respiratory activity. A simultaneous decrease in the reducing-sugar content and the respiratory activity of given lots of roots indicates a correlation between reducing-sugar content and respiration, but seasonal changes and environmental conditions to which the sweet potatoes have been previously subjected tend to obscure any such correlation in different lots. Experiments with wounded roots indicate that the sugar content is not the limiting factor in the respiration of the sweet potato. The reducing sugars are the immediate source of respira-

tory material. The cane sugar is relatively stable in the sweet potato, and when once formed it does not appear to be readily utilized in the process of respiration, while starch and other carbohydrates are present in abundance.

LITERATURE CITED

(1) BORODIN, J.
1876. Physiologische Untersuchungen über die Athmung der beblätterten Sprosse. *In* Arb. St. Petersb. Gesell. Naturf., Bd. 7, p. 1–114, 3 pl. (Russisch.) *Abstract in* Just's Bot. Jahresber., Jahrg. 4, 1876, Abt. 3, p. 919–923. 1878. Original not seen.

(2) ———
1881. Untersuchungen über die Pflanzenathmung. Erste Abhandlung. *In* Mem. Acad. Imp. Sci. St.-Petersb., s. 7, t. 28, no. 4, 54 p., 2 pl.

(3) DELEANO, N. T.
1912. Studien über den Atmungsstoffwechsel abgeschnittener Laubblätter. *In* Jahrb. Wiss. Bot., Bd. 51, Heft 5, p. 541–592.

(4) HASSELBRING, Heinrich, and HAWKINS, L. A.
1915. Physiological changes in sweet potatoes during storage. *In* Jour. Agr. Research, v. 3, no. 4, p. 331–342.

(5) MAIGE, A., and NICOLAS, G.
1910. Recherches sur l'influence des solutions sucrées de divers degrés de concentration sur la respiration, la turgescence et la croissance de la cellule. *In* Ann. Sci. Nat. Bot., s. 9, t. 12, no. 1, p. 315–368.

(6) MAYER, —.
1875. Ueber den Verlauf der Athmung beim keimenden Weizen. *In* Landw. Vers. Stat., Bd. 18, p. 245–279.

(7) MÜLLER, Hermann.
1882. Ueber Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. *In* Landw. Jahrb., Bd. 11, p. 751–828.

(8) PALLADINE, W.
1893. Recherches sur la respiration des feuilles vertes & des feuilles étiolées. *In* Rev. Gén. Bot., t. 5, no. 59, p. 449–473.

(9) ———
1899. Influence de la lumière sur la formation des matières protéiques actives et sur l'energie de la respiration des parties vertes des végétaux. *In* Rev. Gén. Bot., t. 11, no. 123, p. 81–105.

(10) ———
1899. Influence des changements de température sur la respiration des plantes. *In* Rev. Gén. Bot., t. 11, no. 127, p. 241–257.

(11) RISCHAWI, L.
1876. Einige Versuche über die Athmung der Pflanzen. *In* Landw. Vers. Stat., Bd. 19, p. 321–340.

(12) WOLKOFF, A. von, and MAYER, Adolf.
1874. Beiträge sur Lehre über die Athmung der Pflanzen. *In* Landw. Jahrb. Bd. 3, p. 481–527, 4 fig.

CHERRY AND HAWTHORN SAWFLY LEAF MINER

By P. J. PARROTT, *Entomologist*, and B. B. FULTON, *Assistant Entomologist, New York Agricultural Experiment Station, Geneva, N. Y.*

INTRODUCTION

The existence in the State of New York of a leaf miner attacking cherry (*Prunus* spp.) foliage was brought to the attention of the Experiment Station by the receipt of affected foliage during the latter part of June, 1910. An examination of the orchard from which the material had been collected showed that more or less of the leaves on nearly all of the trees of a variety known as English Morello had shriveled and died, while here and there were others with well-defined light-colored areas or blisters, revealing a loss of chlorophyll. Siftings of earth from beneath the trees showed that the causal agent was the larva of a species of sawfly. A number of these were carried through successive stages of development to the following year, when adults were obtained. Some specimens were forwarded to Dr. A. D. MacGillivray, formerly of Cornell University, who reported that the insect represented a new species, the type of a new genus, and should be recorded as *Profenusa collaris*. The information was also given that the creature had been reared from the hawthorn (*Crataegus* spp.).

HOST PLANTS OF SAWFLY LEAF MINER

According to present knowledge, the host plants of the sawfly leaf miner are the cherry and the hawthorn. Of the cherries, it has so far largely confined its attacks to the English Morello variety. It is not commonly observed with the Montmorency or Early Richmond, which would indicate that its presence on these varieties is accidental and occurs when they are grown in proximity to the English Morello. The susceptibility of one fruit and the apparent unattractiveness or resistance to the insect of the other fruits is a curious fact, since all are cultivated varieties of the same cherry, *Prunus cerasus*, and plantings of each kind, growing side by side, may be frequently observed in this State. The two sorts, Montmorency and English Morello, represent groups of cherries which vary more or less in both tree and fruit but have a constant difference only in a single character—the juice in the fruits of one is colorless; in the other it is red. This sharp discrimination on the part of the sawfly leaf miner seems all the more anomalous when considered in the light of its extreme partiality to the foliage of certain hawthorns which are only remotely related to the cherry.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
bi

Vol. V, No. 12
Dec. 20, 1915
N. Y. (Geneva)—4

In its attacks on hawthorns the leaf miner tunnels the foliage in the same manner as that of the cherry. During the course of our studies it has been very evident that the pest is more destructive to certain species of Crataegus than it is to the English Morello cherry. As has been rarely observed in the case of the latter plant, one may find as many as five larvæ mining a single leaf. With hawthorns having a relatively small and narrow leaf, as *C. geneseensis*, there may be an entire destruction of the pulpy tissue, in which event all that remains of the affected leaf is the epidermis, which dries up and ultimately falls to the ground. At the height of an attack, which occurs when the larvæ are reaching maturity, hawthorns which are much infested take on a brownish cast and appear as if struck by a blight or swept by fire. In decorative plantings the destructive work of the insect may assume such a character that the attractiveness of certain species of hawthorns as ornamental shrubs is seriously marred.

About Geneva the sawfly leaf miner is most common in the foliage of an unidentified hawthorn belonging to the Medioximae group, while such species as *C. pedicellata* and *C. punctata*, growing in the immediate vicinity of the former, have so far shown little or no injury and are generally exempt from attack. Dr. C. S. Sargent, Director of the Arnold Arboretum, writes that the insect has become established in the plantings of *Crataegus* spp. and that it is especially destructive to hawthorns of the *crus-galli* group and to *C. nitida*, *C. rotundifolia*, *C. pruinosa*, and other species. Similar conditions exist at the New York Botanical Garden and, as elsewhere, certain species of Crataegus are quite badly infested, while a few species have so far been free from attack.

In the public parks at Rochester, N. Y., notably Genesee Park, the insect has in recent years become a serious pest. Hawthorns representing a wide range of species and grown in extensive numbers feature prominently in certain landscape plantings. In these the sawfly leaf miner has become established, and its destructiveness may be readily observed during May and June. Some haws have been seriously affected, while others have been exempt from injury. Here, again, various hawthorns of the *crus-galli* group have proved to be very susceptible to the pest, and certain species of other groups have shown considerable injury.

DISTRIBUTION OF SAWFLY LEAF MINER.

As a cherry pest the sawfly leaf miner is definitely known to occur in injurious numbers in orchards of English Morello cherry about Geneva in western New York and about Germantown, which is located in the Hudson Valley. It has been reported to the Station as occurring about Schenectady, but the statement of its presence in that locality has not been verified. In view of its occurrence in two communities which are widely separated, it would seem reasonable to suppose that the pest exists in

other localities where sour cherries are extensively grown. However, a careful survey by the orchard and nursery inspectors of the Department of Agriculture in all of the leading fruit-growing counties of the State has failed to find any evidences of the work of the insect except in the foregoing localities. A study of available literature indicates that the insect is not known to occur as a cherry pest outside the State of New York.

As a depredator of hawthorns the sawfly leaf miner has a wider range of distribution. It is known, as already indicated, as a serious pest of hawthorns growing about Boston, Mass., and it is common on various species of Crataegus growing in the vicinity of New York City, Rochester, Ithaca, Geneva, and Skaneateles, all of which are located in the State of New York.

APPEARANCE OF THE INJURY

As implied by its common name, the insect is a leaf-mining species and its work is very characteristic. The injury is first indicated by a small, thin, sinuous channel which finally swells out into a large blister-like area of a light-brown color, resembling that of dead leaf tissues. The attack by the larva of the sawfly leaf miner begins on the edge of the leaf toward the stem and continues along one side toward the leaf apex, the tunnel increasing in dimensions with the growth in size and the progress of the insect. Upon reaching the tip of the leaf the grub reverses its course and works backward toward the stem, consuming the remainder of the pulpy tissues between the main rib and the margin of the leaf. As a result, the parenchyma, or soft cellular tissue, is eaten, leaving the epidermis, which turns brown and forms a large blister. These blisters are very conspicuous on the upper surfaces of the leaves. Oftentimes the whole leaf is mined, but usually with most of the foliage only from one-quarter to one-half of the whole area of a leaf is destroyed. (Pl. LI, fig. 1.) Only the leaves that first unfold are subject to attack, and during some seasons hardly any of these escape the insect's depredations. The principal damage occurs during the last week of May and the early part of June, or about one month before the harvesting of the fruit. With the disappearance of the larvæ the leaves most seriously affected shrivel, die, and finally drop to the ground, causing defoliation, which varies in importance according to the extent of infestation and the influence of seasonal conditions on the rate of growth.

The actual effect of the work of the insect upon the crop is not easily measured and during most years is perhaps not of serious extent. However, as previously indicated, the destructive power of the pest is mainly exercised on the leaves that unfold with the bursting of the buds. In years of slight precipitation and when new growth is of small extent and of slow development the plant is dependent on such foliage as it carries at the time, and any extended injury to it must result in a set-

back, with correspondingly ill effects on the maturing crop of fruit. In years when the production of new growth is more rapid the damage caused by the sawfly leaf miner is of much less importance, as the large leaf surface under the circumstances is sufficient for the needs of the plant, and the loss of affected foliage does not result in an important reduction in leaf area.

The hawthorns are more subject to severe attacks than the cherry, and during some seasons plants may be observed on which there is hardly a leaf that does not show injury. Notwithstanding the partiality of the sawfly leaf miner for this plant, hawthorns seem able to withstand considerable destruction of foliage without marked external evidences of the weakening of the tree. As shown in Plate LI, figure 2, the attractiveness of the plants as ornamental shrubs may be seriously marred.

DESCRIPTION OF LIFE STAGES OF SAWFLY LEAF MINER

EGG

The egg is elliptical in shape, but is not entirely symmetrical in its outline, as one side shows a greater curvature than the other. It is, when removed from surrounding plant tissues, circular in cross section, but in its normal position in the leaf structure it is much flattened, owing to pressure. The chorion is a thin, white, shining, flexible membrane. The measurements of eggs when not compressed are: Length, 0.5 to 0.7 mm.; diameter, 0.28 to 0.36 mm.

LARVA

To determine the number of instars, the mines were carefully examined for all insect remains, when the head molts were collected and measured as to width. The body remnants from some of the molts in first larval instars were occasionally missing, having probably been eaten, but in very few cases were the head structures not in good condition for examination. The width of the head is fairly constant for the first larval instar, but in the more advanced stages there is considerable variation. On the basis of head measurements it appears that the larva normally molts five times in its mine. It finally enters the ground and molts again in transforming to a pupa.

The first five instars have the same general form and differ one from the other principally in size. The body is broadest at the first and second thoracic segments and gradually tapers toward the rear. The thoracic legs are short and conical and are composed of five segments, which include the thick basal and the small hooked terminal structures. All the abdominal segments except the last bear short rounded prolegs on the ventral side. The head is horizontal in the early stages, but slopes downward slightly in later instars. It is broad and flat, rounded on the sides, and obtuse in front. On the dorsal side it bears four longitudinal sutures. The outer pair run back from the ends of the clypeus and divide the head into three almost equal sections. The inner pair extend halfway across the middle section, dividing it into three equal areas. The eyes are wanting. The antennæ are very short and are apparently composed of three segments. The maxillary palpi are large and protrude from beneath the head. The labial palpi are very small. The mandibles are short and thick, deeply hollowed on the inner side, and do not protrude beyond the end of the broadly notched labrum.

The technical description of each of the larval stages follows:

FIRST INSTAR.—Body translucent, white, shining; only slightly wrinkled, and with a green streak, due to alimentary tract, showing plainly in the abdominal segments. Prolegs appear as only slight elevations.

Head is slightly brownish, being of dark color on the outer and posterior edges; mouth parts are reddish brown. The ventral side of the first thoracic segment has a pair of brownish gray marks, shaped roughly like a T, with the cross bar running longitudinally and the perpendicular reaching outward to a point just in front of the leg. A semicircular line of the same color occurs in front of the anus and is interrupted on the median line.

Newly hatched larvæ are about 1.2 mm. in length, and after feeding, the body grows, reaching a length of 2.3 mm. Width of head, 0.36 to 0.42 mm.; average, 0.39 mm.

SECOND INSTAR.—All markings of body are more extensive than in preceding stage. Dorsal side with some specimens has a broad, faint, brownish gray, transverse band on the first thoracic and two spots on the second thoracic segment. The pair of marks on ventral side of first thoracic segment are shaped more like inverted V's, and between them there is a large longitudinal band. The second and third segments have median oval spots. Each proleg is marked by a narrow crescent on the anterior side. A semicircular mark on the last segment extends over half a circle and is not interrupted on the median line.

Length, 2.6 to 3 mm. Width of head, 0.48 to 0.55 mm.; average, 0.52 mm.

THIRD INSTAR.—All markings are the same as in preceding stage, but are much fainter. Prolegs are more prominent; those on the first and penultimate abdominal segments are small.

Length, 3.2 to 4.3 mm. Width of head, 0.63 to 0.73 mm.; average, 0.67 mm.

FOURTH INSTAR.—The characteristic markings in preceding stages practically disappear in this instar. A ring of several rows of minute papillæ surrounds the anus. These probably exist in the earlier instars and escape detection because of their small size.

Length, 4.5 to 7.2 mm. Width of head, 0.8 to 0.9 mm.; average, 0.85 mm.

FIFTH INSTAR.—This is similar to fourth instar. There are no distinct color markings.

Length, 6.5 to 7.5 mm. Width of head, 0.92 to 1.07 mm.; average, 1 mm.

SIXTH INSTAR.—The body does not differ from that of preceding stage. The head assumes a vertical position. The four sutures on the dorsal side are very faint. The clypeus and labrum are shorter than in fifth instar. The mandibles protrude prominently and do not meet at the ends. The labium and maxillæ project from beneath the head to beyond the tips of the mandibles.

Length is same as in fifth instar or may be a trifle shorter. Width of head, 0.90 to 1.05 mm.; average, 1 mm.

PUPA

Until color of adult begins to show, the pupa is white in all portions except the eyes, which are reddish. Length about 5 mm.

ADULT

"Body [of female] black, with the clypeus, labrum, malar space, the mandibles, the first segment of the antennæ, the tegulæ, a narrow margin to the pronotum, and the legs, for the most part, whitish. The prothorax, except the parts named, the cephalic part of the mesopleuræ, and the pectus, rufous; the posterior femora more or less shaded with fuscous; the head smooth with antennal furrows interrupted on the middle of the face; the furrows surrounding the postocellar area deep and distinct, the vertical furrows not reaching the occiput; the median ocellus placed on a flat depression; a pit above the antennal socket; the median fovea minute but dis-

tinct; the clypeus truncate; the first and second antennal segments subequal, the third segment subequal to one and two together and longer than four; the saw-guides with the dorsal and ventral margins converging and the apex bluntly pointed; the male differs in having the rufous part of the thorax inclined to whitish and extending over the entire pleuræ, the venter of the abdomen and a broad band on the lateral part of the dorsal aspect, broader behind, sometimes fused on the meson, whitish; the posterior femora not fuscous. Length 3 to 4 mm."[1]

LIFE HISTORY AND HABITS OF SAWFLY LEAF MINER

EMERGENCE OF ADULTS

From puparia obtained on April 18, 1913, by sifting earth from beneath cherry trees, two male and seven female sawfly leaf miners made their appearance during a period extending from April 28 to May 2. On May 6 six males and six females were obtained in a cherry orchard, and only one of the flies was obtained in cages intended to trap the insects as they emerged from the ground. On May 7 five males and seven females were caught in breeding cages, and at this date the insects were present in large numbers on the trees. The insects continued to appear in the cages, a few each day, until May 19, which for 1913 was the latest date for the emergence of the flies for that year. Observations for several seasons show that the flies make their appearance when the first leaf clusters are unfolding and the cluster buds are beginning to open.

EARLY HABITS

At the time of their emergence from the ground the sawfly leaf miners are fully colored and are very active creatures. They are apparently very susceptible to temperature conditions. If disturbed on cold days, they drop suddenly from the foliage, attempting to fly while in midair. Failing in this effort, they drop to the ground and crawl to some elevated object, on which they renew their attempts to seek flight.

They copulate within less than a day after their appearance from the soil. In this act the male approaches the female backward, so that the tips of their abdomens come in contact while their heads are opposed to each other. Then the male reaches back with the hind legs and grasps the female over the back of her body, placing at the same time the tip of his abdomen under that of the female and inserting the penis under the flap at the base of the ovipositor. The outer flaps of the male genitalia are pressed closely against the under side of the female's body. The whole process is a matter of one to three minutes. One pair contained in an observation jar copulated three times within a space of half an hour.

OVIPOSITION

The females are apparently ready to oviposit soon after they make their escape from the ground. One specimen was dissected about 17 hours after its appearance, and in the ovaries and oviducts there were

[1] MacGillivray, A. D. New genera and species of sawflies. *In* Canad. Ent., v. 46, no. 10, p. 364-365. 1914.

counted 15 fully developed eggs. Another that had been out for two days began to deposit eggs immediately when cherry leaves were introduced into its cage. In the orchard eggs were first found during the year 1913 on May 7; in that season adults were first observed on May 6, although the insects may have been present on the trees for a day or two before and escaped detection. During the first days of the oviposition period one or sometimes two leaves in a cluster may show the presence of eggs. The females seem to manifest a preference for leaves which are first to appear and which are partly folded. The process of oviposition requires only about a minute. Details of this operation proved difficult to determine because of the extreme shyness of the females, which fly quickly on the approach of any object.

The lower surface of the egg lies in contact with the lower epidermis, which has been cut free from the other tissues of the leaf so as to form a small blister-like cavity or pocket. The egg is usually within 1 or 2 mm. from the edge of the leaf; rarely on the extreme edge or more than 3 mm. from the margin. On the upper side at the edge of the cavity there is usually a stoma, through which the ovipositor is probably thrust. An examination of 91 eggs at random shows that they are more often deposited near the base of the leaf than the tip. About 70 per cent of the eggs were in the area of the leaf from one-eighth to one-third the distance from the base, 20 per cent near the middle, and about 10 per cent occurred in the portion of the leaf toward the tip. From 1 to 5 eggs were observed on a single leaf, and the average for all observations was 2.3 eggs per leaf.

HATCHING AND LARVAL ACTIVITIES

During 1913 young larvæ were first observed on May 24 as the trees were coming into full bloom, but judging from the sizes of some of the mines it was evident that a few eggs had hatched one or two days earlier. By May 27 the hatching period was practically completed. In the field it proved difficult to determine the period of incubation, but eggs deposited on cherry leaves in the insectary hatched in eight days from date of oviposition. Under normal conditions incubation would probably extend over a larger number of days.

Upon hatching, the young larva works its way through the tissue of the leaf until it reaches the upper epidermis. It usually mines toward the distal end of the leaf, generally keeping close to the edge and feeding with the ventral side in contact with the upper epidermis. When the tip of the leaf has been reached the creature reverses its course, proceeding along the area adjoining the midrib; or if there is no interference by another larva it may cross over the main rib and tunnel back along the edge of the opposite half of the leaf.

The mine, as viewed from above, during its first stages of development is rather dark brown in color, which is accounted for in part by frass along the edges of the roof of the tunnel. As the affected area increases

in size, especially in its breadth, the mine becomes light brown, while the edges incline to a darker shade. Observed from beneath, the only visible indication of the initial activities of the insect is a small oval spot, which marks the original cavity constructed by the adult for the reception of the egg, and this contains in addition to the shriveled egg membrane accumulations of frass from the early feeding operations of the larva. Later, the underside of the tunnel also becomes brown, with the exposed epidermis wrinkled, but, in general, the destructive work of the insect is not so apparent on the lower as on the upper surface of the leaf.

There is a fairly definite relationship between the size of the mine and the age of the larva with respect to the different instars. In general, mines under 5 mm. long and 2 mm. at their greatest width contain larvæ in the first instar; mines that are 5 by 2 mm. to 12 by 4 mm. contain larvæ in the second instar; mines that are 8 by 5 mm. to 8 by 6 mm. contain larvæ of the third instar; mines that are 18 by 6 mm. to 28 by 8 mm. contain larvæ of the fourth instar; and mines of greater dimensions than the foregoing are occupied by larvæ of the fifth instar.

PUPATION

Upon reaching maturity the larvæ make a hole in the tissues forming the mine, usually the upper epidermis, which forms the roof. From the opening they make their escape to the edge of the leaf, when they drop to the ground. During 1912 the larvæ began to leave the foliage on June 7, and by June 10 it was estimated that 50 per cent of the insects had abandoned their mines. On June 18 it was difficult to find a specimen on the tree, while June 22 was the latest date that any of the insects were seen on the leaves. Upon reaching the ground they bury themselves several inches deep in the soil and construct an earthen cell. The cocoon, which is oval in shape, consists of particles of earth glued together and lined with a cement which renders it impervious to water and strong enough to resist considerable pressure without crushing. The insect passes the winter in the larval stage. However, the pupa begins to form in the fall. Specimens obtained during October showed the developing compound eyes and ocelli, while of examples secured the following April the adult characters of the head could be plainly seen through the skin, and their bodies were decidedly humped. One of these specimens which was kept in a cool room transformed to a pupa on or before April 23. Others obtained from an orchard on May 2, 1913, were all in the pupal stage, and one female pupa was partly colored.

NATURAL ENEMIES OF SAWFLY LEAF MINER[1]

A common enemy of the sawfly leaf miner is the chalcidid *Trichogramma minutum* Riley, which is an egg parasite. During the five years that

[1] Through the courtesy of Dr. L. O. Howard, the identifications of the parasites were made by Messrs. A. A. Girault and A. S. Rohwer, of the United States Bureau of Entomology.

Profenusa collaris has been under observation, *T. minutum* has twice made its appearance in conspicuous numbers in infested cherry orchards, in 1912 and in 1915. During the former year the larger percentage of the eggs of the leaf miner were attacked, and on some trees it was difficult to find an egg-bearing leaf which had not been visited by the parasite. In 1915 parasitism ranged from about 40 to 90 per cent on individual trees. Taking all trees into consideration, of the eggs deposited by the insect a larger percentage of them certainly failed to hatch than hatched, and for this mortality *T. minutum* appeared to be largely responsible.

The parasite was reared from both cherry and hawthorn foliage. The majority of the eggs of the leaf miner that were dissected contained a single parasite, and in only a few instances were twin larvæ or pupæ observed. On June 2, 1915, the parasites were all in the larval state, but on June 5, when the larvæ of *P. collaris* were beginning to abandon their mines in the foliage, about 50 per cent of the parasites were in the pupal state. By June 7 they had nearly all transformed to pupæ, and on June 9 the first adult appeared. During succeeding days the chalcidids appeared in large numbers, and the last specimen to make its appearance emerged on June 14. While the parasite was abundant about Geneva during this year, it was relatively quite scarce on plantings of *Crataegus* spp. at Rochester.

Besides the foregoing parasite there has been reared from *P. collaris* an ichneumon which proved to be a new species and has been listed by Rohwer[1] as "*Pezoporus tenthredinarum.*" Apparently there is associated with this ichneumon an undescribed tryphonine, but owing to the small numbers collected it is impossible to make any definite statement at this time as to its status as a parasite of the sawfly leaf miner.

METHODS OF CONTROL

REMOVAL OF AFFECTED LEAVES

Of the operations systematically practiced, one that will probably prove most effective and economical in controlling the sawfly leaf miner is the picking of affected leaves. This species is peculiarly susceptible to this kind of repressive method, since there is only one brood of larvæ to attack the foliage, and oviposition extends over only a short period. The effect is that hatching of eggs and maturing of larvæ are, practically speaking, almost simultaneous for all of the creatures, and their activities during their injurious stages are therefore restricted to a relatively short period. By careful timing it is possible at a single picking to collect practically all of the larvæ by removing the affected leaves, which should then be burned to destroy the insects therein. The removal and destruction of all mined leaves, coupled with another practice—the destruc-

[1] Rohwer, S. A. Descriptions of new species of Hymenoptera. *In* Proc. U. S. Nat. Mus., v. 49, p. 216. 1915.

tion of wild hawthorns in the immediate vicinity of the cherry orchard should leave few opportunities for the pest to develop to injurious numbers.

FUMIGATION WITH HYDROCYANIC-ACID GAS

Of the various measures employing insecticides tested by this station to protect cherry foliage from the work of the leaf miner, fumigation with hydrocyanic-acid gas alone was effective. Most cherry growers in New York are not equipped with suitable apparatus to undertake this means of affording protection to their trees, and fumigation should only be undertaken as an extreme measure and in an experimental way under expert direction.

CULTIVATION

Cultivation, if done with care and at the proper time, is destructive to many insects with subterranean habits. Species especially that undergo pupal development in the ground are not only peculiarly sensitive to disturbances of the soil, but plowing and cultivation, besides breaking up the cells of hibernating larvæ, exert another detrimental influence, exposing the helpless insects to insectivorous birds and other foes. Since it is the normal habit of the larvæ of this sawfly leaf miner to live in earthen cells for the greater portion of the life cycle of the species, such practices as fall or early spring plowing or cultivation are to be recommended from an entomological standpoint. These measures, fortunately, are standard operations which are invariably practiced by the most successful cherry growers.

DESTRUCTION OF UNCULTIVATED HOST PLANTS

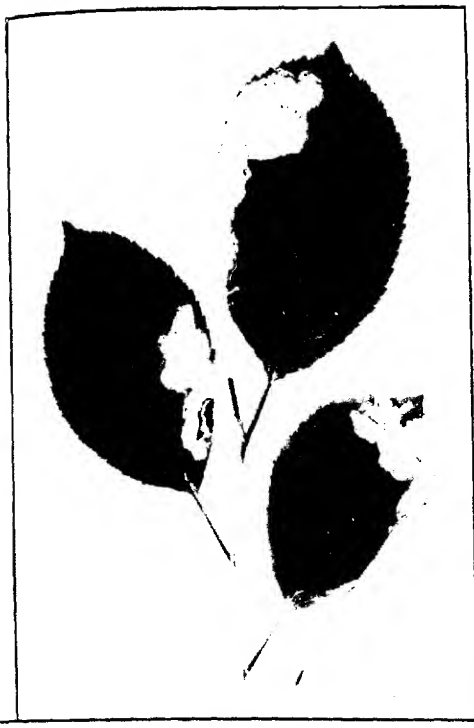
The fact that the sawfly leaf miner is very partial to hawthorns, especially of the group *C. crus-galli*, and breeds most abundantly on them, suggests the desirability of destroying these plants when they exist in the immediate vicinity of a cherry orchard. The value of this operation is not known; but until there is more knowledge of the breeding habits of the pest the removal of wild plants along roadsides and hedgerows that are attractive to the insect for purposes of propagation would appear advisable as a precautionary measure.

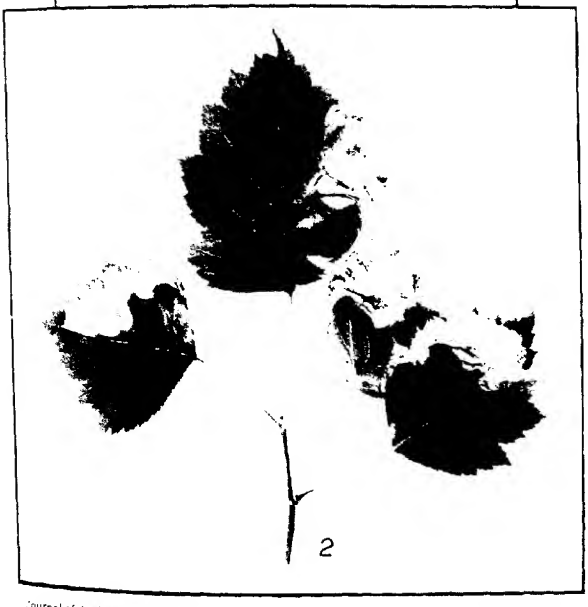
SPRAYING OF HAWTHORNS

For the protection of hawthorns in decorative plantings, spraying seems to be preferred to any of the preceding measures. The insecticide which has given the most satisfactory results is composed of 1 pint of nicotine solution (40 per cent) to 100 gallons of water to which are added 4 pounds of soap. In making the treatment the liquid should be used in liberal amounts and applied with rather high pressures at the time when the insects first begin to mine the foliage.

PLATE LI

Fig. 1.—Leaves of English Morello cherry, showing injury by the sawfly leaf miner.
Fig. 2.—Leaves of hawthorn, showing injury by the sawfly leaf miner.




2

VARIATIONS IN MINERAL COMPOSITION OF SAP, LEAVES, AND STEMS OF THE WILD-GRAPE VINE AND SUGAR-MAPLE TREE

By O. M. SHEDD,[1]

Chemist, Kentucky Agricultural Experiment Station

INTRODUCTION

In a previous publication Kastle and the writer (9)[2] have shown the relation existing between the mineral components of the sap of the wild-grape vine (*Vitis cordifolia*) and those contained in the young leaves and stems at a certain period in its growth during the same year. At that time these writers stated that they did not know whether these relations would hold true throughout the growing season, and they purposed to continue the investigation so as to include the sap and other materials from different portions of this vine and other plants.

Since our former publication, the writer has found in the literature at hand that considerable work has been done by Chandler (1), Harris and Gortner (8), Dixon and others (2, 3, 4, 5, 6, 7) on the physiochemical properties of certain saps or plant juices, but, so far as we have been able to find, no work has been done on the mineral composition of the sap or on the changes occurring therein which might have any bearing on the above-mentioned investigation.

EXPERIMENTS WITH WILD-GRAPE VINE

With this idea in view, the writer has during the last three years (1912–1914) collected samples of the sap from the vine employed in the former work, in order to determine (1) whether the mineral composition of this sap varies at the same time in different parts of the vine, (2) whether it varies during a single season at a certain point, and (3) whether it varies during different years. The analyses are of interest, inasmuch as they show large differences in the composition of the sap, depending on the time and place of collection. The results are given in Tables I to XI and are expressed in percentage by weight, except where otherwise stated. The mineral components of the original sample have been calculated from the amounts found in the ash, except the chlorin, which was determined in the fresh sap. The sulphur-trioxid content of the original substance is probably low, since more or less sulphur is lost in ashing organic materials.

[1] The author desires to express his gratitude to Dr. J. H. Kastle, Director of the Kentucky Experiment Station, for his helpful advice during the progress of this investigation.

[2] Reference is made by number to "Literature cited," p. 541–542.

In order to understand more fully the different tabulations, a brief description of each sample follows.

Nos. 285, 812, and 852 were collected in April of 1912, 1913, and 1914, respectively, from the cut end of the same main branch about 20 feet from the root of the vine and just after the sap flow commenced.

No. 853 was collected in April, 1914, from the cut end of another main branch about 4 feet from the root of the vine and just after the sap flow commenced. This sample was taken at the same time as No. 852.

No. 854 was collected in April, 1914, from the same point as No. 852, but seven days later and just before the sap flow ceased.

No. 900 was collected in April, 1915, from the cut end of one of the main branches about 20 feet from the root of the vine and just after the sap flow commenced. This was a different branch from that from which No. 285 was taken, because no sap exuded from the old branch, and it seemed to have been greatly weakened by the annual loss.

No. 901 was collected in April, 1915, from several of the small branches or shoots which were of the previous year's growth and just after the sap flow commenced. This sample was taken at the same time as No. 900 and from 10 shoots which were located several feet from the main branches.

Nos. 902, 904, and 906 were collected for three successive days from 9 a. m. to 5 p. m., beginning on April 29, 1915, four days after and from the same point as No. 900.

Nos. 903, 905, and 907 were collected for three successive nights from 5 p. m. to 9 a. m., beginning on April 29, 1915, and from the same point as No. 900.

The variation in the percentage composition of the fresh sap and the ash of samples 852, 853, 900, and 901 are given in Tables I and II.

TABLE I.—*Variation in percentage composition of fresh sap collected at the same time from different points on the wild-grape vine* [1]

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Water at 100° C.	99.8279	99.8538	99.8183	99.8431	1 : 1.00	1 : 1.00
Organic matter	.1435	.1112	.1305	.1068	1 : .77	1 : .82
Silica (SiO_2)	.0001	.0001	.0003	.0017	1 : 1.00	1 : 5.67
Ferric and aluminic oxids (Fe_2O_3 + Al_2O_3)	.0001	.0001	.0001	.0004	1 : 1.00	1 : 4.00
Calcium oxid (CaO)	.0160	.0155	.0234	.0268	1 : .97	1 : 1.15
Magnesium oxid (MgO)	.0024	.0025	.0041	.0062	1 : 1.04	1 : 1.51
Sodium oxid (Na_2O)	.0012	.0012	.0010	.0011	1 : 1.00	1 : 1.10
Potassium oxid (K_2O)	.0050	.0112	.0167	.0074	1 : 2.24	1 : .44
Phosphorus pentoxid (P_2O_5)	.0015	.0026	.0030	.0030	1 : 1.73	1 : 1.00
Sulphur trioxid (SO_3)	.0019	.0017	.0025	.0033	1 : .89	1 : 1.32
Chlorin	.0004	.0001	.0001	.0002	1 : .25	1 : 2.00
Total	100.0000	100.0000	100.0000	100.0000		
$d\ \frac{25°}{25°}$	1.0009	1.0008	1.00082	1.00027	1 : 1.00	1 : 3.00
Nitrogen as nitrates	.0013	.0024	.00024	.00001	1 : 1.85	1 : .25
Crude ash	.0384	.0477	.0700	.0602	1 : 1.24	1 : .93

[1] Nos. 852 and 853 were collected in 1914; Nos. 900 and 901 in 1915.

TABLE II.—*Percentage composition of ash of the samples in Table I*

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Silica (SiO_2)	0.339	0.231	0.485	2.505	1 : 0.68	1 : 5.16
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.261	.210	.143	.574	1 : .80	1 : 4.01
Calcium oxid (CaO)	41.628	32.535	33.432	40.386	1 : .78	1 : 1.21
Magnesium oxid (MgO)	6.364	5.296	5.828	9.424	1 : .83	1 : 1.62
Sodium oxid (Na_2O)	3.234	2.522	1.483	1.633	1 : .78	1 : 1.10
Potassium oxid (K_2O)	13.146	23.465	23.787	11.103	1 : 1.78	1 : .47
Phosphorus pentoxid (P_2O_5)	3.860	5.380	4.349	4.543	1 : 1.39	1 : 1.04
Sulphur trioxid (SO_3)	5.008	3.531	3.562	5.052	1 : .71	1 : 1.42
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	73.840	73.170	73.069	75.220	.....	.....

From an examination of Table I it is apparent that the water, calcium, and sodium content of the sap are fairly constant when collected at two different points at the same time during the same year, while the silica, iron, aluminum, potassium, phosphorus, and chlorin are the large variable constituents, depending on the time and point of collection. The organic matter is higher in the sap taken at a point on the main branch about 20 feet from the root than it is on the same branch closer to the ground or on the new branches. The silica, iron, aluminum, calcium, magnesium, and sulphur, however, are higher in the sap in the new branches. These facts agree with the writer's previous findings, which show that the minerals accumulate in the leaves. As the grapevine puts forth leaves every year only on the parts of more recent growth, the above results are what one would naturally expect when considered in connection with the former work.

Another interesting point is that certain constituents—namely, silica, iron, aluminum, magnesium, and phosphorus—may be about the same in the sap when collected from two different points at the same time during a given year, but vary widely when compared the following season.

A further point of interest is that while the ratio of calcium oxid to magnesium oxid is fairly constant in each sap of Table I, that of the potassium oxid to sodium oxid is variable, as shown in Table III.

TABLE III.—*Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of the wild grape collected at the same time from different points on the vine*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852	6.7 : 1	4.2 : 1
853	6.2 : 1	9.3 : 1
900	5.7 : 1	16.7 : 1
901	4.3 : 1	6.7 : 1

TABLE IV.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at different times during the same season* [1]

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902.[b]	Ratio between— Nos. 852 and 854.	Nos. 900 and 902
Water at 100° C.	99.8279	99.7545	99.8183	99.7026	1 : 1.00	1 : 1.00
Organic matter	.1435	.1821	.1305	.2208	1 : 1.27	1 : 1.69
Silica (SiO_2)	.0001	.0003	.0003	.0007	1 : 3.00	1 : 2.33
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0001	.0001	.0001	.0003	1 : 1.00	1 : 3.00
Calcium oxid (CaO)	.0160	.0221	.0234	.0277	1 : 1.38	1 : 1.18
Magnesium oxid (MgO)	.0024	.0036	.0041	.0047	1 : 1.50	1 : 1.15
Sodium oxid (Na_2O)	.0012	.0013	.0010	.0011	1 : 1.08	1 : 1.10
Potassium oxid (K_2O)	.0050	.0277	.0167	.0316	1 : 5.54	1 : 1.89
Phosphorus pentoxid (P_2O_5)	.0015	.0045	.0030	.0069	1 : 3.00	1 : 2.30
Sulphur trioxid (SO_3)	.0019	.0037	.0025	.0036	1 : 1.95	1 : 1.44
Chlorin	.0004	.0001	.0001	.....	1 : .25	.....
Total	100.0000	100.0000	100.0000	100.0000	.....	.....
$d\frac{25°}{25°}$	1.0009	1.0007	1.00082	.....	1 : 1.00	.....
Nitrogen as nitrates	.0013	.0028	.00004	.....	1 : 2.15	.....
Crude ash	.0384	.0863	.0700	.1012	1 : 2.25	1 : 1.45

[1] Nos. 852 and 854 were collected in 1914; Nos. 900 and 902, in 1915.
[b] Composition by volume, but this does not appreciably affect the percentage by weight.

TABLE V. *Percentage composition of ash of samples in Table IV*

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902.	Ratio between— Nos. 852 and 854.	Nos. 900 and 902.
Silica (SiO_2)	0.339	0.371	0.485	0.678	1 : 1.09	1 : 1.40
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.261	.093	.143	.254	1 : .36	1 : 1.78
Calcium oxid (CaO)	41.628	25.627	33.432	27.386	1 : .62	1 : .82
Magnesium oxid (MgO)	6.364	4.225	5.828	4.602	1 : .66	1 : .79
Sodium oxid (Na_2O)	3.234	1.486	1.483	1.078	1 : .46	1 : .73
Potassium oxid (K_2O)	13.146	32.080	23.787	31.198	1 : 2.44	1 : 1.31
Phosphorus pentoxid (P_2O_5)	3.860	5.269	4.349	4.771	1 : 1.37	1 : 1.36
Sulphur trioxid (SO_3)	5.008	4.271	3.562	3.564	1 : .85	1 : 1.00
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	73.840	73.422	73.069	73.531	.....	.....

In Table IV it appears that in both years there is a concentration of practically all the minerals in the sap at the end of the sap flow, or when new leaves develop, compared with the beginning. The ratio of increase of some of the minerals—namely, silica, iron, aluminum, potassium, phosphorus, and sulphur—in one or both years is much greater than the remainder. There is also a wide variation in the percentages of ash in the different samples, which partly accounts for some of these differences (Table V). Furthermore, an examination of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid shows that the former remains fairly constant, while the latter is variable and demon-

strates the large amount of potassium oxid in the sap at the end of the sap flow compared with the beginning, since the sodium oxid is fairly constant during both years. See Table VI.

TABLE VI.—*Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape taken from the same point on the vine at different times during the same season*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852	6.7 : 1	4.2 : 1
854	6.1 : 1	21.3 : 1
900	5.7 : 1	16.7 : 1
902	5.9 : 1	28.7 : 1

An examination of the minimum and maximum percentages of the minerals in the sap collected at the same point during four successive years and just after the sap flow commenced shows the largest variations which have been found (Table VII). The constituents vary in order of magnitude as follows: Potassium, chlorin, iron, aluminum, silica, phosphorus, sulphur, magnesium, sodium, and calcium. Again there is a wide variation in the ash content of the different samples (Table VIII).

TABLE VII.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at the beginning of the sap flow during four successive years*

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum.
Water at 100° C.	99.6340	99.8665	99.8279	99.8183	1 : 1.00
Organic matter	.2782	.0917	.1435	.1305	1 : 3.03
Silica (SiO_2)	.0005	.0005	.0001	.0003	1 : 5.00
Ferric and aluminic oxids ($Fe_2O_3 + Al_2O_3$)	.0006	.0002	.0001	.0001	1 : 6.00
Calcium oxid (CaO)	.0220	.0206	.0160	.0234	1 : 1.46
Magnesium oxid (MgO)	.0044	.0043	.0024	.0041	1 : 1.83
Sodium oxid (Na_2O)	.0017	.0016	.0012	.0010	1 : 1.70
Potassium oxid (K_2O)	.0468	.0112	.0050	.0167	1 : 9.36
Phosphorus pentoxid (P_2O_5)	.0058	.0017	.0015	.0030	1 : 3.87
Sulphur trioxid (SO_3)	.0052	.0016	.0019	.0025	1 : 3.25
Chlorin	.0008	.0001	.0004	.0001	1 : 8.00
Total	100.0000	100.0000	100.0000	100.0000	.....
$d\frac{25°}{25°}$	1.0035	1.00067	1.0009	1.00082	1 : 1.00
Nitrogen as nitrates	.0075	.00048	.0013	.00004	1 : 187.50
Crude ash	.1130	.0570	.0384	.07000	1 : 2.94

TABLE VIII.—*Percentage composition of ash of samples in Table VII*

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum
Silica (SiO_2)	0.405	0.809	0.339	0.485	1 : 2.39
Ferric and aluminic oxids (Fe_2O_3+ Al_2O_3)	.540	.387	.261	.143	1 : 3.78
Calcium oxid (CaO)	19.490	36.070	41.628	33.432	1 : 2.14
Magnesium oxid (MgO)	3.900	7.594	6.364	5.828	1 : 1.95
Sodium oxid (Na_2O)	1.500	2.742	3.234	1.483	1 : 2.18
Potassium oxid (K_2O)	41.380	19.617	13.146	23.787	1 : 3.15
Phosphorus pentoxid (P_2O_5)	5.090	3.059	3.860	4.349	1 : 1.66
Sulphur trioxid (SO_3)	4.590	2.742	5.008	3.562	1 : 1.83
Carbon dioxid, not determined	.....	.....	.....	.....	.....
Total	76.895	73.020	73.840	73.069	.....

As stated before, Nos. 285, 812, and 852 were collected from the same branch, whereas No. 900 was taken an equal distance from the root on another branch, as the former was so greatly weakened that no sap exuded from it at the proper time, although new growth came on it later, showing that it was not dead. If a comparison now be made of Nos. 285, 812, and 852, it will be found that there has been a marked reduction in practically all of the mineral substances in the sap in the two succeeding years compared with the first, and, moreover, this was very sharp in some constituents in the second and, in others, in the third year. Furthermore, it will be noticed that among those which show a decided decrease in the second year are potassium and phosphorus, both of which are included among the chief essential plant-food elements.

According to the different analyses of the sap, potassium is among the high mineral constituents, and as this element has shown the largest loss, this may account for the weakened condition of the branch.

The ratios of calcium oxid to magnesium oxid and of potassium oxid to sodium oxid in the various samples of Table VII are as given in Table IX.

TABLE IX.—*Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape from the same point on the vine at the beginning of the sap flow during four successive years*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
285	5.0 : 1	27.5 : 1
812	4.8 : 1	7.0 : 1
852	6.7 : 1	4.2 : 1
900	5.7 : 1	16.7 : 1

Table IX shows that the ratio of calcium oxid to magnesium oxid is fairly constant in the different samples, while the wide variation in the potassium oxid and sodium oxid from 27.5 in 1912 to 4.2 in 1914 would indicate that these figures were obtained from the sap of different plants rather than from that of the same vine at different times.

TABLE X.—*Variation in percentage composition*[1] *of fresh sap of wild grape collected for three successive days and nights*[2]

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Water at 100° C.	99.7026	99.7354	99.7436	99.7473	99.7592	99.7469	
Organic matter	.2208	.1971	.1766	.1892	.1732	.1874	1 : 1.00
Silica (SiO_2)	.0007	.0006	.0008	.0007	.0005	.0007	1 : 1.27
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0003	.0002	.0005	.0001	.0005	.0001	1 : 1.60
Calcium oxid (CaO)	.0277	.0248	.0248	.0245	.0228	.0233	1 : 5.00
Magnesium oxid (MgO)	.0047	.0042	.0089	.0049	.0070	.0045	1 : 1.21
Sodium oxid (Na_2O)	.0011	.0008	.0039	.0008	.0041	.0014	1 : 2.12
Potassium oxid (K_2O)	.0326	.0279	.0296	.0239	.0245	.0254	1 : 5.13
Phosphorus pentoxid (P_2O_5)	.0069	.0060	.0077	.0056	.0054	.0067	1 : 1.32
Sulphur trioxid (SO_3)	.0036	.0030	.0036	.0030	.0028	.0036	1 : 1.43
Chlorin, not determined							1 : 1.29
Total	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	
Crude ash	.1012	.0916	.0925	.0843	.0780	.0839	1 : 1.30

[1] By volume.
[2] Nos. 902, 904, and 906 were collected on successive days; Nos. 903, 905, and 907 were collected on successive nights.

TABLE XI.—*Percentage composition of ash of samples in Table X*

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Silica (SiO_2)	0.678	0.691	0.884	0.804	0.641	0.788	1 : 1.38
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.254	.267	.590	.079	.641	.110	1 : 8.11
Calcium oxid (CaO)	27.386	27.068	26.820	29.034	29.167	27.796	1 : 1.09
Magnesium oxid (MgO)	4.602	4.621	9.607	5.840	8.939	5.366	1 : 2.09
Sodium oxid (Na_2O)	1.078	.823	4.220	1.006	5.269	1.672	1 : 6.40
Potassium oxid (K_2O)	31.198	30.478	31.986	28.277	31.430	30.232	1 : 1.13
Phosphorus pentoxid (P_2O_5)	6.771	6.544	8.271	6.604	6.950	7.999	1 : 1.26
Sulphur trioxid (SO_3)	3.564	3.261	3.841	3.524	3.628	4.327	1 : 1.33
Carbon dioxid, not determined							
Total	75.531	73.753	86.219	75.168	86.665	78.290	

Referring to the results in Table X, it will be seen that there is a considerable variation occurring daily in the mineral composition of the sap and that, as a rule, most of its constituents are present in larger amounts during the day, while, on the other hand, its composition is more constant at night (Table XI).

As there was such a wide variation in short periods of time in the composition of the sap of this vine, it was thought desirable to collect further samples of the young leaves and stems in order to determine if this would hold true in regard to these parts. Accordingly, in June, 1915, or two months after the sap was first collected, and every two weeks thereafter for six weeks, samples of the succulent young stems and leaves representing the same stage of growth were taken. Therefore, the results are somewhat comparable with each other and with those formerly obtained, since the earlier samples were taken in a similar manner in Nos. 908 and 909. The consecutive analyses are given in Tables XII to XV along with those of Nos. 627 and 628 of 1912.

TABLE XII.—*Variation in percentage composition of young green leaves of wild-grape vine in the same and in different years*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Water at 100° C.	75.4700	75.1200	71.1975	73.1525	72.6015	1:1.06
Organic matter	22.8500	23.3181	26.8851	25.3750	25.9097	1:1.18
Silica (SiO_2)	.1372	.0514	.0642	.0291	.0537	1:4.71
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0214	.0197	.0394	.0117	.0165	1:3.37
Calcium oxid (CaO)	.7200	.5478	.7585	.5515	.4427	1:1.71
Magnesium oxid (MgO)	.1337	.1112	.1333	.1004	.1078	1:1.33
Sodium oxid (Na_2O)	.0356	.0167	.0214	.0256	.0287	1:2.13
Potassium oxid (K_2O)	.3427	.5619	.5943	.5076	.5752	1:1.73
Phosphorus pentoxid (P_2O_5)	.2260	.2104	.2020	.1891	.1992	1:1.20
Sulphur trioxid (SO_3)	.0634	.0428	.1043	.0595	.0650	1:2.44
Total	100.0000	100.0000	100.0000	100.0000	100.0000	.....
Crude ash	2.3300	2.0140	2.4595	1.9530	1.8790	1:1.31

TABLE XIII.—*Percentage composition of ash of samples in Table XII*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Silica (SiO_2)	5.890	2.550	2.610	1.490	2.860	1:3.95
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.920	.980	1.600	.600	.880	1:2.67
Calcium oxid (CaO)	30.900	27.200	30.840	28.240	23.560	1:1.31
Magnesium oxid (MgO)	5.740	5.520	5.419	5.143	5.737	1:1.12
Sodium oxid (Na_2O)	1.530	.827	.870	1.209	1.527	1:1.85
Potassium oxid (K_2O)	14.710	27.899	24.163	25.992	30.613	1:2.08
Phosphorus pentoxid (P_2O_5)	9.700	10.447	8.215	9.682	10.600	1:1.29
Sulphur trioxid (SO_3)	2.720	2.127	4.239	3.046	3.457	1:1.99
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	72.110	77.550	77.956	75.402	79.234	.....

TABLE XIV.—*Variation in percentage composition of young green stems of wild-grape vine in the same and in different years*

Constituent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Water at 100° C.	79.2500	84.2750	81.9385	83.3210	82.6645	1 : 1.06
Organic matter.	20.0437	14.8654	17.0893	15.7397	16.4453	1 : 1.35
Silica (SiO_2)	.0041	.0048	.0069	.0037	.0040	1 : 1.86
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0003	.0051	.0038	.0041	.0032	1 : 17.00
Calcium oxid (CaO)	.1114	.1558	.2244	.2488	.1792	1 : 2.23
Magnesium oxid (MgO)	.0346	.0539	.0642	.0567	.0557	1 : 1.86
Sodium oxid (Na_2O)	.0171	.0078	.0133	.0158	.0093	1 : 2.19
Potassium oxid (K_2O)	.3883	.4813	.5154	.4846	.5098	1 : 1.33
Phosphorus pentoxid (P_2O_5)	.1277	.1055	.1078	.1010	.1009	1 : 1.27
Sulphur trioxid (SO_3)	.0228	.0454	.0364	.0246	.0281	1 : 1.99
Total	100.0000	100.0000	100.0000	100.0000	100.0000	.....
Crude ash	1.0200	1.1490	1.2810	1.3790	1.2175	1 : 1.35

TABLE XV.—*Percentage composition of ash of samples in Table XIV*

Constitutent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Silica (SiO_2)	0.400	0.420	0.540	0.270	0.330	1 : 2.00
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.030	.440	.300	.300	.260	1 : 14.67
Calcium oxid (CaO)	10.920	13.560	17.520	18.040	14.720	1 : 1.74
Magnesium oxid (MgO)	3.390	4.694	5.013	4.115	4.578	1 : 1.48
Sodium oxid (Na_2O)	1.680	.679	1.039	1.145	.764	1 : 2.47
Potassium oxid (K_2O)	38.070	41.892	40.241	35.140	41.876	1 : 1.19
Phosphorus pentoxid (P_2O_5)	12.520	9.184	8.419	7.322	8.291	1 : 1.71
Sulphur trioxid (SO_3)	2.240	3.951	2.840	1.784	2.305	1 : 2.21
Carbon dioxid, not determined	.....	.....	.....	.....	.....	.....
Total	69.250	74.820	75.912	68.116	73.124	.....

In Tables XII and XIV it will be found that the ratio between the lowest and the highest result obtained for each of the other mineral constituents in the several samples of leaves and stems is more constant than it is for the silica, iron, and aluminum. It will further be found that the results obtained this year on the different samples corroborate, except in one instance, those of 1912 in showing that there is a concentration of all the constituents in the leaf compared with the stem, and the exception is that whereas the potassium content of the stem in 1912 was greater than in the leaf, this year (1915) it was less in every case.

In the leaf the silica, sodium, magnesium, and phosphorus are uniformly lower than in 1912 and the organic matter and potassium are higher, while the other constituents vary both above and below the former results. In the stem, however, the organic matter, sodium, and phosphorus are lower than formerly and the iron, aluminum, calcium, magnesium, potassium, and sulphur are higher, while the silica is variable.

Another interesting point is that most of the results show that the mineral constituents are lower in the leaves of this year (1915) than formerly, while in the stem they are higher.

The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the leaf and stem below show, as did those of the sap, that the former is more constant than the latter (Table XVI).

TABLE XVI.—*Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in leaves and stems of young wild-grape vine in the same and in different years*

Part and sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
Leaf:		
627	5.4 : 1	9.6 : 1
908	4.9 : 1	33.6 : 1
910	5.7 : 1	27.8 : 1
912	5.5 : 1	21.5 : 1
914	4.1 : 1	20.0 : 1
Stem:		
628	3.2 : 1	22.7 : 1
909	2.9 : 1	61.7 : 1
911	3.5 : 1	38.8 : 1
913	4.4 : 1	30.7 : 1
915	3.2 : 1	54.8 : 1

EXPERIMENTS WITH SUGAR MAPLE

Having found such a wide variation in the composition of the sap of the wild-grape vine, it was thought that it might prove of further interest to compare the analyses of the sap of the same sugar-maple tree (*Acer saccharum*) collected during two successive years. Accordingly, early in 1913 and 1914, just after the sap began to rise, samples were collected at the same point on the tree, about 3 feet from the ground.

Also, for a further comparison, the sap was collected in 1913, just after the sap flow commenced, from a water-maple tree (*Acer saccharinum*) at a point about 10 feet from the ground.

The results are given in Tables XVII and XVIII.

TABLE XVII.—*Variation in composition of the sap of the water-maple and sugar-maple trees*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio between Nos. 776 and 851.
		No. 776.[a]	No. 851.[b]	
Water at 100° C.	98.2035	98.2953	98.3227	1 : 1.00
Organic matter	1.7677	1.6812	1.6247	1 : .97
Silica (SiO_2)	.0013	.0016	.0011	1 : .69
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.0001	.0001	.0001	1 : 1.00
Calcium oxid (CaO)	.0053	.0097	.0200	1 : 2.06
Magnesium oxid (MgO)	.0009	.0018	.0026	1 : 1.44
Sodium oxid (Na_2O)	.0020	.0004	.0009	1 : 2.25
Potassium oxid (K_2O)	.0118	.0084	.0178	1 : 2.12
Phosphorus pentoxid (P_2O_5)	.0023	.0007	.0060	1 : 8.57
Sulphur trioxid (SO_3)	.0004	.0002	.0033	1 : 16.50
Chlorin	.0047	.0006	.0008	1 : 1.33
Total	100.0000	100.0000	100.0000	
$d_{25°}^{25°}$	1.0056	1.0045	1.0059	1 : 1.00
Nitrogen as nitrates	.0007			
Crude ash	.0296	.0336	.0678	1 : 2.02

[a] Collected in 1913 just after the sap flow commenced.
[b] Collected in 1914 just after the sap flow commenced, from same point on the same tree as No. 776.

TABLE XVIII.—*Percentage composition of ash of samples in Table XVII*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio between Nos. 776 and 851.
		No. 776.	No. 851.	
Silica (SiO_2)	4.444	4.868	1.701	1 : .35
Ferric and aluminic oxids ($Fe_2O_3+Al_2O_3$)	.506	.237	.220	1 : .93
Calcium oxid (CaO)	18.003	28.864	29.561	1 : 1.02
Magnesium oxid (MgO)	2.926	5.399	3.893	1 : .72
Sodium oxid (Na_2O)	6.751	1.241	1.352	1 : 1.09
Potassium oxid (K_2O)	39.945	24.902	26.211	1 : 1.05
Phosphorus pentoxid (P_2O_5)	7.651	2.079	8.881	1 : 4.27
Sulphur trioxid (SO_3)	1.238	.518	4.834	1 : 9.33
Carbon dioxid, not determined				
Total	81.464	68.108	76.653	

In Table XVII we find that the calcium, magnesium, sodium, potassium, phosphorus, and sulphur are much higher in the sugar-maple sap in 1914 than in 1913 and the silica is lower, while the water, organic matter, iron, and aluminum are about the same in both years. The largest varying constituents are sulphur and phosphorus.

Again, on comparing the sap of the sugar maple with that of the water maple, there are found large differences in the calcium, magnesium, sodium, potassium, phosphorus, sulphur, and chlorin, while the water, organic matter, silica, iron, and aluminum are about the same.

The large amount of sodium and chlorin in the sap of the water maple may be explained as due to the fact that this tree was located on a city lot and may have received sodium chlorid from the drainage, while the sugar maple was located in the country. On the other hand, the wild-grape vine was also on a city lot, but in a different locality, and its sap did not show a large chlorin content; still, this may have been due to the difference in the drainage of the two places.

The differences obtained in the mineral constituents of the several samples of sap can not be due altogether to the different moisture content of the soil, for the large variations in the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the tables show that it is not a dilution of the sap by the soil water.

The moisture content of the soil at the time of the sap collection was not determined, and, of course, this would be influenced by several factors, such as temperature, rainfall, sunshine, and wind at that period. Taking into account the rainfall alone will not explain the differences obtained, as will be seen from Table XIX.

TABLE XIX.—*Rainfall in inches during four successive years*

Month.	1912.	1913.	1914.	1915.
January	1.78	10.35	2.50	4.38
February	2.50	2.61	3.87	1.12
March	4.36	6.04	2.24	1.49
April	6.89	2.41	2.23	.65

During the spring of this year (1915) there was less rainfall in this vicinity than for years, and there is no doubt that the moisture content of the soil at the time of the sap collection in 1915 was considerably lower than it was the three preceding years. If the results are to be explained from the dilution standpoint, then those of Nos. 285 and 900 in Table VII should be in harmony with what has just been stated, while, as a matter of fact, they are contradictory, except for one constituent.

The foregoing results show that the sap has a variable mineral composition which later on influences the structure of the growing parts, and this undoubtedly explains the differences in composition of the same and different varieties of plants.

SUMMARY

(1) There is considerable variation in the composition of the sap of the wild-grape vine when collected at the same time from two different points. This has been the case for two seasons.

(2) Large differences in the composition of this sap were found when it was collected at the same point on the vine at different times during the same season. The minerals in the sap are higher at the end of the

sap flow than at the beginning. This has also been proved for two seasons.

(3) The widest variations in the composition of this sap were found when it was collected at the same point on a main branch of the vine at the beginning of the sap flow during four successive years. The periodic loss of sap greatly weakened this branch, and there was also a steady decline in the mineral components of the sap taken from it, particularly potassium and phosphorus.

(4) There was found a considerable variation occurring daily in the composition of this sap. The mineral constituents were generally higher during the day and the sap had a more uniform composition during the night.

(5) The young leaves and stems of this vine at the same stage of growth were also found to vary considerably in composition during different years and also in the same season.

(6) The sap of the same sugar-maple tree was found to vary widely in composition when collected at the same point on the tree during two successive years just after the sap flow had commenced.

(7) The mineral composition of the sap of the water-maple tree was found to be different from that of the sugar maple.

(8) The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid, together with other factors, demonstrate that the differences in composition can not be altogether explained as being due to a dilution of the sap from the water in the soil.

(9) It has been shown that the sap has a variable mineral composition which influences the structure of the growing parts and undoubtedly explains the differences in composition of the same and different varieties of plants.

LITERATURE CITED

(1) CHANDLER, W. H.
1914. Sap studies with horticultural plants. Mo. Agr. Exp. Sta. Research Bul. 14, p. 489–552, 13 pl. Bibliography, p. 535–539.

(2) DIXON, H. H.
1914. Changes produced in the sap by the heating of branches. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 14, no. 15, p. 224–228.

(3) ——
1914. Transpiration and the Ascent of Sap in Plants. . . 216 p., illus. London.

(4) —— and ATKINS, W. R. G.
1910. On osmotic pressure in plants; and on a thermo-electric method of determining freezing points. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 12, no. 25, p. 275–311, 2 fig.

(5) —— ——
1912. Variations in the osmotic pressure of the sap of Ilex aquifolium. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 13, no. 18, p. 229–238, 2 fig.

(6) —— ——
1912. Variations in the osmotic pressure of the sap of the leaves of Hedera helix. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 13, no. 19, p. 239–246, 1 fig.

(7) DIXON, H. H., and ATKINS, W. R. G.
1913. Osmotic pressures in plants. II.—Cryoscopic and conductivity measurements on some vegetable saps. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 13, no. 29, p. 434-440. Bibliography, p. 440.

(8) HARRIS, J. A., and GORTNER, R. A.
1914. Researches on the physico-chemical properties of vegetable saps. 2. Note on a comparison of the physico-chemical constants of the juice of apples and pears of varying size and fertility. *In* Biochem. Bul., v. 3, no. 10, p. 196-201, pl. 2.

(9) SHEDD, O. M., and KASTLE, J. H.
1912. On the composition of the ash of the sap, leaves and young stems of the wild grape vine (Vitis cordifolia). *In* Jour. Amer. Chem. Soc., v. 34, no. 10, p. 1415-1424.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

10 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

▽